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Lectins, Lectin Genes, and Their Role in Plant Defense

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REVIEW

INTRODUCTION

Lectins are carbohydrate-binding proteins that bind glycans of glycoproteins, glycolipids, or polysaccharides with high affinity (Goldstein and Hayes, 1978). Because of their binding specificity, they have the capability to serve as recognition molecules within a cell, between cells, or between organisms. It is assumed that lectins play fundamental biological roles in plants because they are found in many different species and in many different organs and tissues.

Of the many plant lectins that have been characterized extensively, most are secretory proteins, meaning that they enter the secretory system and subsequently accumulate either in vacuoles or in the cell wall and intercellular spaces. For example, the well-known lectins phytohemagglutinin, concanavalin A, soybean agglutinin, pea lectin, and favin are all present at relatively high levels and accumulate in vacuoles in the cotyledons (1% to 8% of total protein), and at lower levels in the embryonic axes of the seeds. These lectins are synthesized during seed development together with the more abundant seed storage proteins. During germination and seedling growth, both storage proteins and lectins are broken down to provide amino acids for the growing seedling. Lectins are also often quite abundant in vegetative plant organs such as roots, leaves, rhizomes, and stems. Some of these are vacuolar while others such as the chitin-binding Datura seed lectin are extracellular. Vacuolar lectins also occur in cereal seeds, but are much less abundant (1 μg/dry grain) and occur only in specific cell layers of the embryo (e.g., wheat germ agglutinin in the coleorhiza and rootcap of the wheat embryo) (see Etzler, 1986, for a discussion of plant lectins).

In this review, we advance the idea that lectins have evolved through gene duplication and divergence, and that the carbohydrate binding domains of lectins have become incorporated into families of proteins whose members play important roles in plant defense. Although many roles have been proposed for plant lectins (Etzler, 1986), we believe that the most likely function for the vacuolar lectins is in plant defense. Although we emphasize the defense role of lectins, it is clear that extracellular root lectins may be involved in the recognition of bacteria (*Rhizobium* and *Bradyrhizobium* sp) for the purpose of establishing symbioses, as first proposed by Bohlool and Schmidt (1974). Root lectins are an important determinant of host specificity (Diaz et al., 1989); however, their role may not be in the actual species-specific recognition, which appears to be mediated by small fatty acylated and sulfated tetrasaccharides (Lerouge et al., 1990); rather, lectins may serve to agglutinate large numbers of bacteria at the roothair surface. Their role in establishing a symbiosis may have evolved from the ability of lectins to agglutinate and immobilize bacteria as a defense reaction.

There are a number of ways by which vacuolar lectins can interact with molecules within and outside the cell. First, when dry seeds imbibe water, vacuolar proteins and especially lectins are released into the imbibition water (Fountain et al., 1977). This results in the presence of lectins in the vicinity of the germinating seed, where they can interact with potential pathogens. Second, when seeds or other plant organs are eaten by predators, lectins will be released from the disrupted cellular structures of the plant tissues. These lectins will then come in contact with the glycoproteins that line the intestinal tracts of the predators, possibly inhibiting absorption of nutrients. Third, when fungal hyphae grow into plant tissues, they may disrupt cellular compartmentation, causing the release of vacuolar lectins that may inhibit further hyphae growth.

Evidence for an evolutionary relationship among the lectins is provided by the high degree of amino acid sequence identity shared by the different legume lectins. In addition, one plant species may contain structurally related lectin proteins that have different biological properties. For example, the castor bean contains two distinct but structurally related lectins with different biological properties: ricin and *Ricinus communis* agglutinin. Ricin is highly cytotoxic but is a weak agglutinin, whereas *R. communis* agglutinin is weakly cytotoxic but a strong agglutinin.

In this review, we will examine two specific cases of homology among lectin proteins. We have chosen these two examples because recent work clearly establishes the

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following two principles we wish to illustrate: (1) the evolution of lectin genes within one species, and (2) the evolution of lectin genes by the incorporation of carbohydrate binding domains into proteins of many plant species. First, we will compare the four known members of the phytohemagglutinin (PHA) family of polypeptides present in the common bean *Phaseolus vulgaris*. The four homologous polypeptides of this family (PHA-E, PHA-L, α-amylase inhibitor, and arcelin) have different biological or plant defense properties.

Second, we will examine a family of proteins whose members all have a chitin binding domain as part of their structures. This domain of 43 amino acids is the basic building block of wheat germ agglutinin (WGA), the lectin of wheat (*Triticum aestivum*), and of the homologous lectins from barley and rice. This chitin binding domain is also found in several other proteins that have plant defense properties, including hevein, nettle lectin, and several chitinases. These proteins are present in widely divergent plant species.

GENES AND PROTEINS IN THE PHYTOHEMAGGLUTININ FAMILY

The common bean contains PHA, an abundant hemagglutinin and mitogen that has been thoroughly characterized (Goldstein and Hayes, 1978). This tetrameric lectin is composed of five isoforms of the polypeptides PHA-E and PHA-L in different combinations. Tetramers of PHA-E (M_r = 34,000) agglutinate erythrocytes, whereas tetramers of PHA-L (M_r = 32,000) agglutinate leucocytes and have mitogenic activity. Mixed tetramers can bind to both types of blood cells. PHA-E and PHA-L both recognize terminal galactose residues on complex glycans of mammalian glycoproteins. The PHA-E and PHA-L polypeptides are encoded by two tandemly linked genes, *dlec1* and *dlec2*, respectively (Hoffman and Donaldson, 1985). These genes, which are 90% homologous at the nucleotide level, encode proteins that have 82% identity in their amino acid sequences. The biosynthesis, post-translational modifications, and transport to the vacuole of PHA have been studied in considerable detail.

Several years before Hoffman and Donaldson (1985) reported the derived amino acid sequences of PHA-E and PHA-L, the same group (Hoffman et al., 1982) isolated a cDNA that encodes a 27-kD "lectin-like" protein. The presence of 2 methionine residues in the derived amino acid sequence indicated that this cDNA did not encode PHA because all amino acid analyses of purified PHA showed a complete absence of methionine residues. This lectin-like protein is synthesized on the rough endoplasmic reticulum, glycosylated, and transported to the protein storage vacuoles, where it undergoes proteolytic processing to polypeptides of M_r 15,000 to 18,000 (Ceriotti et al., 1989). Work from our (M.J.C.) laboratory has shown that this protein is identical with an already characterized α-amylase inhibitor (αAI) from bean (Moreno and Chrispeels, 1989). When we expressed the cDNA obtained by Hoffman et al. (1982) in transgenic tobacco with a seed-specific promoter, we obtained tobacco seeds with αAI protein and activity (Altabella and Chrispeels, 1990). Because this lectin-like gene encodes the bean αAI, we now refer to this gene as *αai*. The coding sequence of the *αai* gene is 82% homologous to *dlec1* and *dlec2*, the genes that encode the PHA polypeptides. We obtained genomic clones indicating physical linkage between *αai*, *dlec1*, and *dlec2* (P.E. Staswick and M.E. Chrispeels, unpublished data). Thus, *αai* probably arose through the duplication and divergence of an ancestral lectin gene. We do not know whether αAI is a lectin, but this seems unlikely because amino acid sequence comparisons of αAI with PHA-E and PHA-L show that a critical portion of the carbohydrate binding domain of PHA is missing from αAI, as shown in Figure 1. It is important to note that the bean αAI is not at all similar, except in its biological activity, to the α-amylase inhibitors found in cereals.

Another polypeptide in the phytohemagglutinin family was found when Osborn and coworkers examined a protein that is abundantly present in certain Mexican accessions of the common bean that are resistant to the two bean weevils (*Zabrotes subfasciatus* and *Acanthoscelides obtectus*) that are predators of domesticated cultivars. These resistant wild accessions all contain the protein arcelin that is absent from susceptible varieties. Cloning of the cDNA of arcelin-1 (one of the four electrophoretic variants of arcelin) revealed a nucleotide sequence that is 78% homologous to PHA and a derived amino acid sequence with 58% to 61% identity with PHA-E, PHA-L, and αAI (Osborn et al., 1988) (Figure 1). Genetic evidence shows that in these wild accessions the gene for arcelin is tightly linked to the genes for PHA (Osborn et al., 1986). Interestingly, PHA occurs in 90% of all bean cultivars and in all wild accessions, whereas arcelin is absent from cultivated beans and occurs only in 10% of the wild accessions. It appears that all domesticated lines may have come from wild lines that did not have arcelin or that the arcelin gene was lost or inactivated in the course of domestication.

PLANT DEFENSE PROPERTIES OF PROTEINS IN THE PHYTOHEMAGGLUTININ FAMILY

When eaten raw, the common bean has long been known to be toxic to a variety of animals, and the toxicity of purified PHA toward mammals (Pusztai et al., 1979; Jaffé and Vega Lette, 1986, and references therein) and birds (Jayne-Williams and Burgess, 1974) has been demonstrated in feeding trials. Several groups have shown that

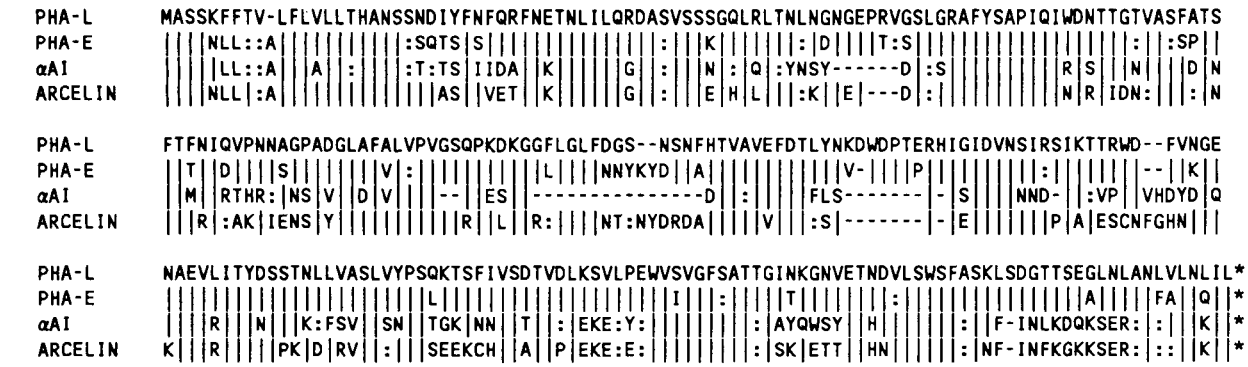

Figure 1. Sequence Similarity of Proteins in the Phytohemagglutinin Family.

PHA-L (Hoffman and Donaldson, 1985) is used as a reference. Aligned amino acid sequences are PHA-E (Hoffman and Donaldson, 1985), αAI (Hoffman et al., 1982), and arcelin (Osborn et al., 1986). Identical amino acids are marked by vertical lines and conservative substitutions by two dots. Gaps introduced for alignment of homologous regions are indicated by dashes.

PHA binds to the intestinal mucosa of rats, resulting in the appearance of lesions, disruptions, and abnormal development of the microvilli (see Liener, 1986, for a review). When PHA is added to the diet of experimental animals, it inhibits the absorption of nutrients across the intestinal wall and greatly increases the bacterial colonization of the small intestine. That PHA is also toxic to insects and specifically to bruchid beetles was first suggested by Janzen et al. (1976) and later apparently confirmed by Gatehouse et al. (1984). Both found that "purified" PHA inhibited the development of larvae of *Callosobruchus maculatus*, the cowpea weevil. However, the results obtained by Gatehouse et al. (1984) showed that impure PHA was more effective than pure PHA in arresting larval development. The impure PHA preparation obtained from a commercial supplier contained polypeptides of M_r 15,000 to 18,000, indicating that it was probably contaminated with α-amylase inhibitor. New results (Murdock et al., 1990) show that rigorously purified PHA is not toxic to the cowpea weevil, and that the inhibitory effect of the less purified preparation used by Gatehouse et al. (1984) was due to the presence of αAI. This latter protein is quite toxic to bruchid beetles (see below).

Arcelin, the PHA-like protein present in some wild accessions of bean, has been shown to be toxic to the bean weevil *Z. subfasciatus* (Osborn et al., 1988). These investigators made a number of crosses between an arcelin-containing wild line of bean and an arcelin-minus cultivated line and showed a dosage relationship between the presence of two, one, or no copies of the *Arc* gene and the emergence of adult bruchid beetles from seeds. In an *arc/arc* genotype, 93% of the larvae emerged as adults; this number dropped to 20% to 40% for *Arc/arc* genotypes, and was 2% for *Arc/Arc* genotypes. In feeding experiments with artificial seeds, 10% (w/w) arcelin-1 in the mixture reduced the percentage emergence of adult *Z. subfasciatus* weevils from 76% to 18%. Lower levels of arcelin (5% w/w) had no effect. Although the arcelin preparation used in these experiments may also have been contaminated with αAI, as was the case with PHA preparations used in earlier studies with bruchids (see above), bean αAI has relatively little effect on the α-amylase present in the midgut of *Z. subfasciatus* (Ishimoto and Kitamura, 1989). Thus, contamination of arcelin with αAI is an unlikely explanation for the observed inhibition of larval development by arcelin.

The fourth protein in the PHA family, αAI, is a powerful inhibitor of mammalian and insect α-amylases. When this protein was fed to insect larvae in an artificial seed system, 0.2% (w/w) was enough to inhibit development of *C. maculatus* larvae quite strongly (Ishimoto and Kitamura, 1989). We observed 100% mortality of *C. maculatus* larvae when αAI accounted for 0.8% of the total protein in artificial seeds (J.E. Huesing, R.E. Shade, M.J. Chrispeels, and L.L. Murdock, manuscript submitted).

The genes for the proteins in the phytohemagglutinin family (PHA-E, PHA-L, αAI, and arcelin) are all linked. These homologous genes presumably arose through duplication and divergence of an ancestral gene. This enabled the different proteins to acquire different plant defense properties. Some of the proteins in this family bind carbohydrates, whereas others have acquired different biological properties, such as inhibition of α-amylase. The plant defense properties of the proteins in the PHA family are summarized in Table 1.

THE CHITIN-BINDING FAMILY OF PROTEINS

The seeds of many species of the Gramineae contain lectins, which specifically bind the sugar *N*-acetylglucosamine (GlcNAc), its oligomers, and chitin, a polymer of

Table 1. Plant Defense Properties of Proteins in the PHA and Chitin-Binding Families

	Property
Phytohemagglutinin family	
PHA-E	Toxic to mammals and birds
PHA-L	Toxic to mammals and birds
αAI	Toxic to weevils
Arcelin	Toxic to weevils
Chitin-binding family	
WGA	Toxic to weevils, European corn borer, and Southern corn rootworm
Rice lectin	Toxic to weevils
Datura lectin	Toxic to weevils
Tomato lectin	Toxic to weevils
Nettle lectin	Toxic to weevils, inhibitory to fungi
Hevein	Inhibitory to fungi
Chitinase	Inhibitory to fungi

GlcNAc residues (see Goldstein and Hayes, 1978, for review). In hexaploid wheat (*T. aestivum*), the GlcNAc-binding lectin WGA consists of three unique isolectins (A, B, and D) encoded by homologous genes within the respective diploid genomes. The cDNA clones encoding isolectins A, B, and D show 93% to 95% identity at the amino acid level (Wright and Raikhel, 1989) and at the nucleotide level (Raikhel and Wilkins, 1987; Smith and Raikhel, 1989a).

Lectins very similar to WGA by immunological, biochemical, and sugar binding criteria are also present in rye (*Secale cereale*) (Peumans et al., 1982a), barley (*Hordeum vulgare*) (Peumans et al., 1982a), rice (*Oryza sativa*) (Tsuda, 1979), couch grass (*Agropyrum repens*) (Cammue et al., 1985), and false brome grass (*Brachypodium sylvaticum*) (Peumans et al., 1982b). Work from our (N.V.R.) laboratory has shown that cDNA clones encoding barley lectin (Lerner and Raikhel, 1989) and rice lectin (Wilkins and Raikhel, 1989) are also homologous to WGA. Barley lectin and WGA-B share 95% amino acid sequence identity, whereas rice lectin and WGA-A share 78% sequence identity. The amino acid sequence identity between rice lectin and WGA-B and WGA-D is only 44% and 33%, respectively, indicating considerable divergence during the course of evolution.

Gramineae lectins are synthesized as preproproteins; their hydrophobic signal peptides are cotranslationally removed and they are modified by the addition of a high mannose glycan on the carboxyl-terminal propeptide (Mansfield et al., 1988; Smith and Raikhel, 1989b). The polypeptides pass through the Golgi complex before accumulation in vacuoles. During transport or after arrival in the vacuoles, the glycosylated carboxyl-terminal propeptide is removed from the proprotein to yield the mature lectin. Recently, our (N.V.R.) laboratory has shown that the carboxyl-terminal propeptide is necessary for correct sorting of these lectins to the vacuole (Bednarek et al., 1990). WGA and barley lectin are 36-kD dimers composed of two identical 18-kD subunits. In cultivated rice species, the majority of the 18-kD subunits undergo an additional proteolytic cleavage event that yields two subunits of 8 kD and 10 kD (Stinissen et al., 1984). The mature proteins of all Gramineae lectins that have been studied consist of four homologous domains of 43 amino acids, as shown in Figure 2. Amino acid sequence comparisons show that the four domains of WGA have 49% to 67% amino acid identity, and the four domains of barley lectin have 48% to 72% amino acid identity, indicating that the genes arose through the duplication of a single domain. This domain has become known as the hevein domain because there is about 50% amino acid sequence identity between these cereal lectin domains and hevein, a small chitin-binding protein found in the latex of the rubber tree *Hevea brasiliensis* (Walujono et al., 1975), as shown in Figure 3.

Recent experiments from our (N.V.R.) laboratory have shown that the cDNA clone that encodes hevein is actually much larger than the nucleotide sequence necessary to encode a 43-amino acid protein (Broekaert et al., 1990) (Figure 2). The hevein cDNA encodes a protein that consists of a putative signal sequence and a hevein domain followed by an additional 144 amino acids. The difference in polypeptide length between purified hevein and the hevein polypeptide deduced from the cDNA clone suggests that hevein is synthesized as a preproprotein that is proteolytically processed (H. Lee and N.V. Raikhel, manuscript in preparation) (Figure 2, arrows). The entire prohevein derived amino acid sequence is homologous (65% to 68%) to the deduced amino acid sequences of two wound-inducible genes of potato (Stanford et al., 1989). Thus, the hevein and the wound-induced genes encode polypeptides consisting of a signal peptide, a hevein domain, and a carboxyl-terminal domain of 133 to 144 amino acids. It is particularly noteworthy that the same gene structure is found in the basic chitinases from bean (Broglie et al., 1986), tobacco (Shinshi et al., 1987), poplar (Parsons et al., 1989), potato (Laflamme and Roxby, 1989), and also in a bifunctional protein (α-amylase inhibitor/chitinase) in the seeds of Job's Tears (*Coix lachryma jobi*) (Ary et al., 1989) (Figure 2).

Another member of the same family of proteins that have a chitin binding domain (Chapot et al., 1986) as part of their structure is the small lectin (UDA, *Urtica dioica* agglutinin or nettle lectin) abundantly present in the rhizomes of the stinging nettle (Peumans et al., 1983). This chitin-binding protein has high specificity toward GlcNAc oligomers, and its cDNA encodes a protein that is much larger than expected (D.R. Lerner and N.V. Raikhel, unpublished data).

Several other chitin-binding lectins isolated from tomato (*Lycopersicon esculentum*) (Kilpatrick, 1980), the greater

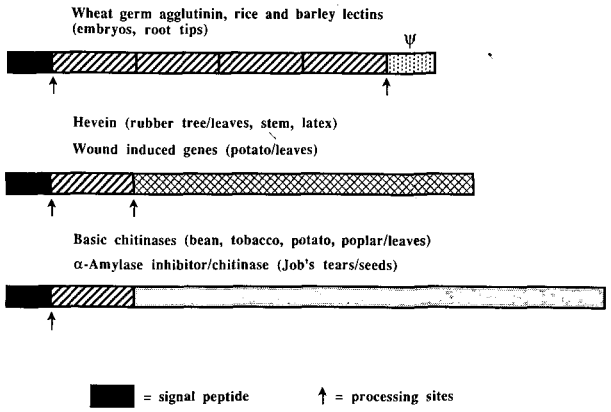

Figure 2. Schematic Representation of Prepropolypeptides with the Chitin Binding Domains.

The preproprotein of wheat germ agglutinin, rice, and barley lectins (Raikhel and Wilkins, 1987; Lerner and Raikhel, 1989; Smith and Raikhel, 1989a; Wilkins and Raikhel, 1989) consists of a signal sequence (dark box) that is cotranslationally cleaved (arrow), four homologous domains of 43 amino acids (hatched boxes), and a 15-amino acid (wheat and barley lectins) or 26-amino acid (rice lectin) carboxyl-terminal domain (dotted box), which contains an *N*-linked high mannose glycan. Before or concomitant with deposition of mature lectins in the vacuole, the glycosylated carboxyl-terminal propeptide is cleaved to yield the mature polypeptide of 18 kD. The mature 18-kD rice lectin undergoes another post-translational cleavage to yield polypeptides of 10 kD and 8 kD (not shown). The deduced amino acid sequence of hevein contains a signal sequence (dark box) that is cotranslationally cleaved (arrow) (H. Lee and N.V. Raikhel, manuscript in preparation), followed by an amino-terminal region containing 43 amino acids (hatched box) (Walujono et al., 1975; Broekaert et al., 1990) and a carboxyl-terminal extension of 144 amino acids (cross-hatched box) (Broekaert et al., 1990). The 187-amino acid proprotein is post-translationally cleaved to yield a mature 43-amino acid hevein and a 144-amino acid carboxyl-terminal polypeptide (H. Lee and N.V. Raikhel, manuscript in preparation). The deduced amino acid sequence of wound-induced genes from potato (Stanford et al., 1989) shows a high degree of homology to both domains of hevein; however, nothing is known about post-translational modifications of these proteins.

The deduced amino acid sequence of basic chitinases from bean (Broglie et al., 1986), tobacco (Shinshi et al., 1987), poplar (Parsons et al., 1989), and potato (Laflamme and Roxby, 1989) share a similar structure: signal sequence (black box), amino-terminal domain (bean, tobacco, and potato, 42 amino acids; poplar, 39 amino acids) (hatched box) and carboxyl-terminal extension (bean, tobacco, 259 amino acids; potato, 258 amino acids; and poplar, 252 amino acids) (stippled box). The partial amino acid sequence for α-amylase inhibitor/chitinase from Job's Tears indicates homology to basic chitinases (Ary et al., 1989). The mature basic chitinases do not undergo post-translational cleavage (Broglie et al., 1986; Shinshi et al., 1987).

celandine (*Chelidonium majus*) (Peumans et al., 1985), thorn-apple (*Datura stramonium*) (Broekaert et al., 1987), and potato (*Solanum tuberosum*) (Allen et al., 1978; Desai et al., 1981) have amino acid profiles similar to hevein and WGA (high glycine and cysteine content). The potato and thorn-apple lectins also contain an unrelated hydroxyproline-rich domain (Allen et al., 1978; Broekaert et al., 1987).

Although genes for tomato, celandine, potato, and thorn-apple lectins have not yet been isolated, the recent cloning of hevein, UDA, the basic chitinase cDNA clones, and the wound-induced genes from potato and poplar makes it clear that the chitin binding domain is a building block (Figure 2) of many proteins, all of which have similar sugar binding specificities. The presence of this common domain suggests that these proteins have evolved as a result of a gene duplication (Gramineae lectins), by gene fusion of the chitin binding domain with unrelated domains (hevein, chitinases, etc.), or both (Figure 2). It is likely that other combinations of the chitin binding domain with other structural domains will soon be found in other plants.

PLANT DEFENSE PROPERTIES OF PROTEINS WITH CHITIN BINDING DOMAINS

The proteins that bind chitin have all been found to affect the growth of organisms that contain chitin (fungi and insects). Gramineae lectins are present in small amounts (Mishkind et al., 1980; Raikhel et al., 1984), but their local concentration is relatively high because they accumulate in specific cell layers of embryonic and seedling tissues (i.e., radicle, coleorhiza, coleoptile, scutellum, root tips) (Mishkind et al., 1982; Raikhel et al., 1984). The specific accumulation of the Gramineae lectins in tissues that establish direct contact with the environment during embryo germination and seedling growth has long been interpreted as evidence for their role in protecting the plant against fungal infections. As early as 1975, Mirelman et al. (1975) proposed that WGA plays a role in defense of seedlings against fungal attack. They observed that WGA inhibits spore germination and hyphal growth of *Trichoderma viride*. These findings have been confirmed by others, but there is disagreement as to whether the observed effects are due to WGA itself or to contaminating chitinases in WGA preparations that are made with chitin affinity columns. Schlumbaum et al. (1986) demonstrated that in vitro, chitinase-free preparations of WGA do not inhibit fungal growth, whereas chitinase does inhibit fungal growth (Table 1).

The effect of WGA on the growth of cowpea weevil larvae was recently demonstrated by Murdock et al. (1990). Using an artificial seed system, they showed that 1% (w/w) WGA increased the time of larval development from 32 days to 55 days, and that all three isolectins of

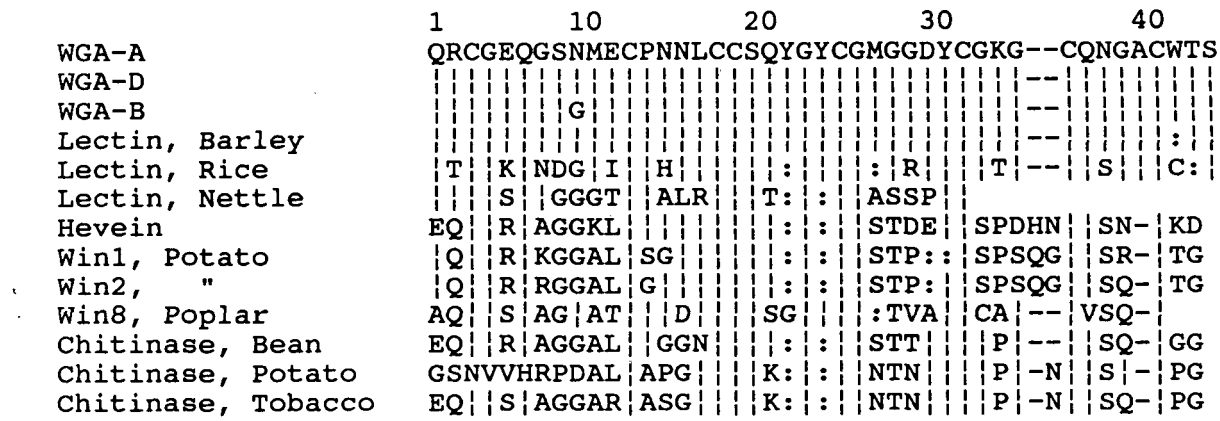

Figure 3. Sequence Similarity of Chitin Binding Domains in the Family of Chitin-Binding Proteins.

Domain A of WGA-A (Wright and Olafsdottir, 1986; Smith and Raikhel, 1989a) is used as a reference. Aligned amino acid sequences are domain A of WGA-D (Wright and Olafsdottir, 1986; Smith and Raikhel, 1989a), domain A of WGA-B (Raikhel and Wilkins, 1987), domain A of barley lectin (Lerner and Raikhel, 1989), domain A of rice lectin (Wilkins and Raikhel, 1989), nettle lectin (Chapot et al., 1986), amino-terminal domain of hevein (Walujono et al., 1975; Broekaert et al., 1990), deduced amino acids of amino-terminal domains of wound-induced genes of potato (Win1 and Win2) (Stanford et al., 1989) and from poplar (Win8) (Parsons et al., 1989), amino-terminal domain of chitinase isolated from bean (Lucas et al., 1985; Broglie et al., 1986), and deduced amino acids of amino-terminal domains of chitinases isolated from tobacco (Shinshi et al., 1987) and potato (Laflamme and Roxby, 1989). Identical amino acids are marked by vertical lines and conservative substitutions by two dots. Gaps introduced for alignment of homologous regions are indicated by dashes.

WGA had similar effects (Huesing et al., 1991). Similar results have now been obtained with rice lectin and UDA (J.E. Huesing, L.L. Murdock, and R.E. Shade, manuscript submitted). However, purified thorn-apple and tomato lectins have significantly less effect on developing cowpea weevil larvae (L.L. Murdock and J.E. Huesing, personal communication). Recently, it has also been demonstrated in in vitro experiments that WGA has an inhibitory effect on development of two important maize pests, the European corn borer (*Ostrinia nubilalis*) and Southern corn rootworm (*Diabrotica undecimpunctata*) (Czapla and Lang, 1991). These investigators have shown that 0.059% WGA (w/w) incorporated into an artificial diet produces 50% mortality of European corn borer larvae (Table 1). In addition, 0.3% WGA (w/w) in the diet had a 50% reduction in weight of the Southern corn rootworm (T.H. Czapla, personal communication).

Although the mode of action of these chitin-binding lectins is not known, it is tempting to speculate that their deleterious effects on insect development is mediated by binding to chitin in the peritrophic membrane that lines the midgut of insects. Such binding could interfere with the uptake of nutrients. The plant defense properties of other Gramineae lectins, potato, and greater celandine lectins, and the α-amylase inhibitor/chitinase obtained from the seeds of Job's Tears have not yet been determined.

In addition to having insect antinutrient activity, the smaller chitin-binding lectin UDA has been shown to have strong anti-fungal properties against several chitin-producing fungi (Broekaert et al., 1989) (Table 1). Because UDA is also purified with chitin columns, it was important to show that the observed effects were not due to contaminating chitinases that also readily bind to these columns and inhibit fungal growth. With some fungi (*Botrytus cinerea*) UDA is more effective than chitinase, whereas with other fungi (*Trichoderma hamatum*) the reverse is true (Broekaert et al., 1989).

The inhibitory effect of hevein (a 43-amino acid protein) on fungal growth has also been demonstrated (Van Parijs et al., 1990) (Table 1). Hevein has 3 times to 5 times less anti-fungal activity than UDA, and 1 order of magnitude less activity than tobacco chitinase with the saprophytic fungi. However, hevein inhibits the growth of the plant pathogenic fungi *B. cinerea*, *Fusarium oxysporum*, *Pyrenophora tritici-repentis*, and others at concentrations of 300 μg/mL to 500 μg/mL, whereas tobacco chitinase is ineffective at 1 mg/mL (Van Parijs et al., 1990).

The addition of chitinase to the fungal medium causes a lysis at the hyphal tips, whereas hevein and UDA result in the formation of thick hyphae, confirming that the two proteins have a different mode of action. Broekaert et al. (1989) suggested that these small chitin-binding proteins cross-link the chitin, preventing cell expansion at the tip of growing hyphae. This binding could slow hyphal growth, acting as the first line of an integrated plant defense system.

CONCLUSION

The evidence we have presented shows two types of evolutionary relationships among proteins with lectin properties. It appears that the PHA genes of the common bean evolved by duplication and divergence from an ancestral gene (all the genes are still linked) and the resulting proteins acquired different biological properties. This is probably also the case for other legumes that contain structurally related proteins, some or all of which have agglutinating properties. The chitin binding domain characteristic of WGA has apparently become incorporated into genes that now encode a variety of proteins in widely divergent plants. In addition, the cereal lectins with their tandem repeats apparently arose through duplication of this domain. All of these proteins have biological functions related to plant defense against chitin-containing pathogens and predators. This combination of fusion and duplication of defense genes may have given plants an evolutionary advantage by creating new proteins with diverse specificities.

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