Are Lectins Involved in Plant-Fungus Interactions?

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Many plants contain proteins or glycoproteins that selectively agglutinate particular types of cells. These agglutinins are called "lectins," and their specificities are due to a complementarity of their combining sites for particular carbohydrate residues. The carbohydrate specificities of the lectins vary among different species of plants (for reviews, see references 17, 22, and 24). The ability of lectins to "recognize" particular carbohydrate residues has made them valuable tools for use in studies of complex carbohydrates in solution and on the cell surface. Indeed, within the last decade, use of these molecules has increased to such an extent that the term lectin has become almost a "household word" among biological scientists.

Despite the vast literature on lectin structures and specificities and the multitude of uses that have been found for them, very little is known about what physiological function they may serve in the plant. Roles postulated for plant lectins include: storage proteins, transportation or trapping of particular carbohydrate residues (5), attractants for specific strains of *Rhizobium* needed for nitrogen fixation in leguminous plants (18), mitogenic stimulators of plant embryonic cells (20), involvement in cell wall extension (21), and various protective functions (5) including plant-fungus interactions (27). The latter postulated role is the subject of this presentation and, as implied by the title of this paper, the physiological involvement of lectins in plant-fungus interactions is still very much an open question. This paper reviews the studies that have suggested the possible role of lectins in plant-fungus interactions and attempts to clarify some of the factors that must be taken into account before this question can be answered. Further, it describes the results of some of the research in our laboratory that may eventually help to clarify the role of lectins in plants.

LECTIN-FUNGUS INTERACTIONS

The ability of lectins to distinguish various types of cells suggested that they may protect plants against various pathogens (5). This hypothesis is attractive because of the specificity of some plant-pathogen interactions. Mirelman et al (27) tested this possibility by studying the ability of the wheat germ lectin (WGA) to interact with *Trichoderma viride*. WGA has a specificity for chitin oligomers of β-$(1\rightarrow4)$-N-acetyl-D-glucosamine (2,25). Using fluorescein isothiocyanate-conjugates of this lectin, Mirelman et al (27) found that WGA could bind to the hyphal tips and septa of *T. viride*. This binding, which was inhibited by chitotriose, did not occur in the mature regions of the hyphae where the chitin may not be as accessible due to overlayering by glucans. These same authors found that WGA inhibits growth and spore germination of this fungus and found evidence that it interferes with chitin synthesis.

In an extension of this work, Barkai-Golan et al (3) found that WGA could bind to the hyphal tips and septa and young spores of a number of species of chitin-containing fungi in the Zygomycetes, Ascomycetes, Basidiomycetes, and Deuteromycetes. The soybean lectin, which recognizes D-galactose and N-acetyl-D-galactosamine (23,29), and the peanut lectin, specific for D-galactose (28), bound to young spores and mature regions of hyphae of *Penicillium* and *Aspergillus* species. All three lectins also were found to interfere with growth and to delay spore germination of *Aspergillus ochraceus*. It was proposed that the lectins may protect plants

against fungal pathogens during the imbibition, germination, and early growth of the seedlings (3,27).

In addition to the above studies, Callow (7) reported that the potato lectin, with a specificity similar to that of WGA (1), will inhibit hyphal extension and spore germination in *Botrytis cinerea*, and Galun et al (15) have shown that WGA binds to the hyphal walls of three mycobionts isolated from lichen fungi.

Although the studies mentioned above show that some lectins can specifically interact with the surfaces and inhibit the growth of some fungi, it must be recognized that these experiments were conducted in the presence of free, soluble lectins thus ensuring the accessibility of the lectin to the fungal surfaces. Whether such accessibility of lectins to fungi occurs in vivo is not yet known. Thus, the ability of lectins to distinguish particular types of fungi may not be related to the physiological role of lectins in plants anymore than is their ability to distinguish particular erythrocytes, and caution should be used in interpreting the significance of these studies. This same criticism is applicable to many of the studies attempting to test whether lectins attract *Rhizobium* spp. Before one can establish the physiological role(s) of lectins it is thus important to have more information on their distribution and cellular localization during the life cycle of the plant.

LECTIN STUDIES AT UC-DAVIS

Distribution of *Dolichos biflorus* lectin. To obtain some insight on the role(s) of lectins in plants, we set out to study the development and distribution of the lectin of *Dolichos biflorus* during the life cycle of the plant. *D. biflorus* (horse gram) was chosen for study because we already had extensively characterized the specificity and structure of the lectin from its seeds (8–10,13,19). The *D. biflorus* lectin can selectively agglutinate
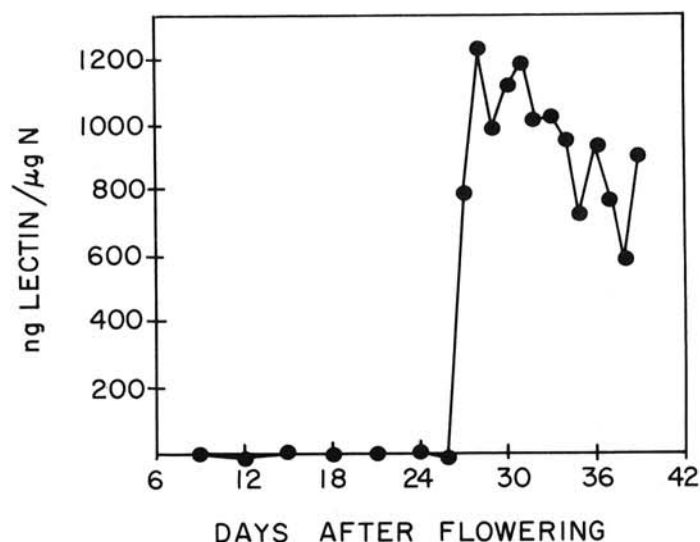

Fig. 1. Lectin content of developing seeds of *Dolichos biflorus* plant. At various times after flowering, the two to six seeds in a pod were pooled, homogenized, and assayed for lectin and N. Each point is the average of data from seeds of two to six pods except for days 29, 35, 36, and 37 when the seeds from only one pod were assayed. (Reprinted from Plant Physiology 61:847-850 [32]. Copyright 1978 American Society of Plant Physiologists.)

blood group A erythrocytes (4) and precipitate blood group A substance (6) due to its specificity for terminal nonreducing α-N-acetyl-D-galactosamine residues (13,19). As with many other lectins, the *D. biflorus* lectin is particularly abundant in the seeds; it constitutes about 9% of the total nitrogen of the seed extract (13,32).

Using a sensitive, competitive radioimmunoassay based on the ability of lectin to inhibit the precipitation of radiolabelled lectin by antibodies to the lectin, we followed the distribution of the *D. biflorus* lectin during the life cycle of the plant. No lectin was detected in the seeds during the first 26 days after flowering, but on the 27th day a large amount was found. The maximum level was reached by 28 days after flowering (Fig. 1), and this level was maintained in the dried seeds. Dissection of germinating seeds at various time intervals showed the lectin was confined to the cotyledons (32) where it has recently been found to be localized in the storage granules or protein bodies (M. E. Etzler et al, *unpublished*). During the absorption of the cotyledons, the lectin level decreased at about the same rate as the other cotyledon reserves (Fig. 2) (32).

No lectin was detected in the roots of the plant throughout its life cycle. Although the stems and leaves initially were thought to contain some lectin (32), these tissues were found instead to contain a glycoprotein that cross-reacts with antibodies against the seed lectin (32,33). Thus, the lectin characteristic of the seeds was confined to the cotyledons during the life cycle of the plant. Lectins in some other plants have been found to have similar distributions (30,31).

Material cross-reactive to antibodies against seed lectin. The material cross-reactive (CRM) to antibodies against the seed lectin was isolated from the stems and leaves of the *D. biflorus* and was found to be a glycoprotein with amino acid and carbohydrate compositions resembling those of the seed lectin (33). The seed lectin is a tetramer composed of two types of subunits (I and II) that appear to differ only at their COOH-terminal ends (9,10,14). The CRM appears to be a dimer composed of a subunit with electrophoretic mobility identical to that of subunit I of the seed lectin and another subunit that has a higher molecular weight than either of the seed lectin subunits (Fig. 3) (33). Both CRM subunits have identical NH$_2$-terminal sequences and these sequences are identical to the NH$_2$-terminal sequences of the seed lectin subunits except for an aspartic acid instead of an asparagine at the second

residue (33). Therefore, both the CRM and the seed lectin subunits may be modifications of a common polypeptide chain.

The CRM did not agglutinate type A erythrocytes or bind to blood group A substance under the normal conditions for testing the seed lectin, and it was postulated that perhaps the CRM was a precursor to the seed lectin that might be activated by proteolytic conversion of the heavy subunit of CRM to subunit II of the seed lectin (33).

Recently the CRM was found to have carbohydrate-binding properties under conditions of low ionic strength but its specificity differs somewhat from that of the seed lectin (11). The CRM is therefore a "lectinlike"glycoprotein and its activity may be under some type of regulatory control in the plant.

By using antibodies against the CRM in a fluorescence microscopy study and in cell fractionation experiments, we found a major portion of the CRM to be associated with the cell walls. This CRM could be released from the cell walls by treatment with cellulase and pectinase (12).

After an epidemic caused by *Erysiphe pisi* broke out in our greenhouse, the levels of CRM appeared to be greatly increased in the cell walls studied by fluorescence microscopy. Infection of the plants with *Botrytis cinerea* (a broad-spectrum fungal plant pathogen), infestation of them with *Pseudomonas phaseolicola* (a bacterial nonpathogen), and wounding the plants all produced similar elevation in CRM (16). These results, however, must still be considered as preliminary; radioimmunoassay of extracts of infected or wounded tissues failed to show significant increase in the level of CRM as compared to that in control tissue extracts. It is possible that the increased CRM may become activated and be susceptible to degradation during extraction; however, as yet, there is no evidence to support this idea.

The above results raise the possibility that the CRM may be the functional lectin in the plant and that the seed lectin may be simply a degradation product of the CRM used as food for the plant during germination. Assuming that the CRM is indeed the functional molecule, it is tempting to speculate that this glycoprotein may function in cell wall elongation as proposed by Kauss and Glaser (21) or in maintaining the integrity of the cell wall in response to stress.
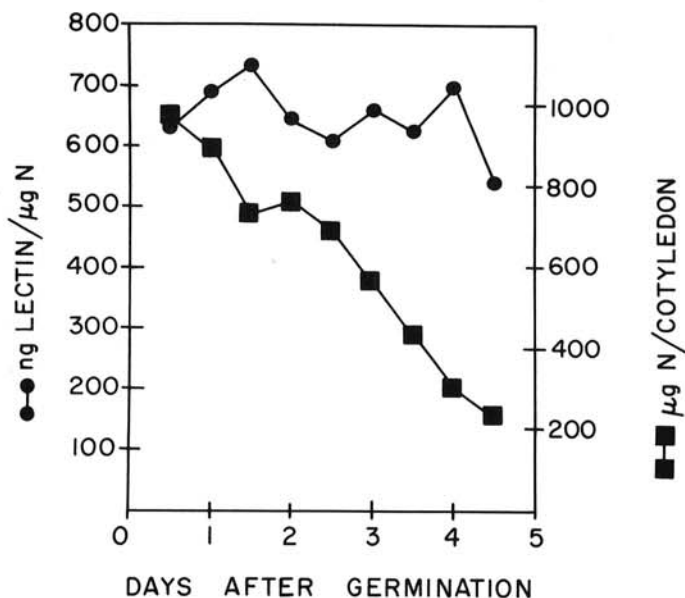

Fig. 2. Lectin content of *Dolichos biflorus* cotyledons during germination of seeds. At 12-hr intervals from the time of planting, cotyledons were homogenized and assayed for N and lectin. ($\bullet$) = nanograms of lectin per microgram of N; ($\blacksquare$) = total amount of N in cotyledon. Each point is the average of 12 assays. (Reprinted from Plant Physiology 61:847-850 [32]. Copyright 1978 American Society of Plant Physiologists.)
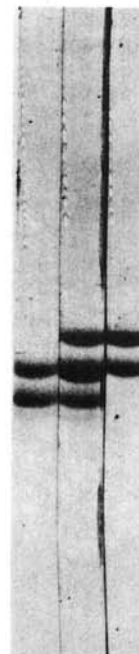

Fig. 3. Discontinuous polyacrylamide gel electrophoresis of cross-reactive material (CRM) and seed lectin isolated from *Dolichos biflorus* and placed on pH 9.7 glycine gels in the presence of 0.1% sodium dodecyl sulfate and 8.0 M urea. The gel at left shows seed lectin subunits; the gel at right shows CRM subunits; the middle gel shows a mixture of CRM and seed lectin subunits. Gels are stained with Coomassie Brilliant Blue.

Although the role of the CRM in the plant is not yet known, there is an indication that this component is not unique to the *D. biflorus* plant. Using immunodiffusion, Mialonier et al (26) found a substance in the leaves of the *Phaseolus vulgaris* plant that cross-reacted with antibodies to the seed lectin of the plant but had a lower molecular weight and did not agglutinate erythrocytes. Further investigations of the properties of these cross-reactive materials and of their relationships to the seed lectins may be of importance in determining the role(s) of lectins in plants.

CONCLUSION

Although some lectins have been found to bind to some fungi and inhibit fungal growth and delay spore germination, it has not yet been established that lectins have a physiological role in protecting plants against pathogenic fungi. Confirmation of the natural role(s) of lectins must await further information on the distribution of lectins during the life cycle of the plant.

During such a distribution study, we discovered a glycoprotein in the stems and leaves of the *D. biflorus* plant that cross-reacts with antibodies against the seed lectin. A major portion of this CRM is present in the cell walls, and under conditions of low ionic strength it is activated and can bind to carbohydrate. Preliminary results indicate that fungal infection or wounding may elevate the level of CRM in the cell wall and it is possible that the CRM may play some role in maintaining the integrity of the cell wall in response to such stress. These studies emphasize the importance of obtaining more information on the distribution and properties of lectins in plants before their physiological role(s) can be determined.

LITERATURE CITED

1. Allen, A. K., and Neuberger, A. 1973. The purification and properties of the lectin from potato-tubers, a hydroxyproline-containing glycoprotein. Biochem. J. 135:307-314.
2. Allen, A. K., Neuberger, A., and Sharon, N. 1973. The purification, composition and specificity of wheat-germ agglutinin. Biochem. J. 131:155-162.
3. Barkai-Golan, R., Mirelman, D., and Sharon, N. 1978. Studies on growth inhibition by lectins of penicillia and aspergilli. Arch. Microbiol. 116:119-124.
4. Bird, G. W. G. 1952. Relationship of the blood sub-groups A, A_2 and A,B, A_2 B to haemagglutinins present in the seeds of *Dolichos biflorus*. Nature 170:674.
5. Boyd, W. C. 1963. The lectins: their present status. Vox Sang. 8:1-32.
6. Boyd, W. C., and Shapleigh, E. 1954. Specific precipitating activity of plant agglutinins (lectins). Science 119:419.
7. Callow, J. A. 1977. Recognition, resistance and the role of plant lectins in host-parasite interactions. Adv. Bot. Res. 4:1-49.
8. Carter, W. G., and Etzler, M. E. 1975. Isolation, characterization, and subunit structures of multiple forms of *Dolichos biflorus* lectin. J. Biol. Chem. 250:2756-2762.
9. Carter, W. G., and Etzler, M. E. 1975. Isolation and characterization of subunits from the predominant form of *Dolichos biflorus* lectin. Biochemistry 14:2685-2689.
10. Carter, W. G., and Etzler, M. E. 1975. Isolation and characterization of cyanogen bromide fragments and a glycopeptide from the *Dolichos biflorus* lectin. Biochemistry 14:5118-5122.
11. Etzler, M. E., and Borrebaeck, C. 1980. Carbohydrate binding activity of a lectin-like glycoprotein from stems and leaves of *Dolichos biflorus*. Biochem. Biophys. Res. Commun. 96:92-97.
12. Etzler, M. E., Gibson, D., and Scates, S. 1979. Localization of inactive form of lectin in primary cell walls of leaves and stems of *Dolichos biflorus*. Plant Physiol. Suppl. 63:134.
13. Etzler, M. E., and Kabat, E. A. 1970. Purification and characterization of a lectin (plant hemagglutinin) with blood group A specificity from *Dolichos biflorus*. Biochemistry 9:869-877.
14. Etzler, M. E., Talbot, C. F., and Ziaya, P. R. 1977. NH_2-terminal sequences of the subunits of *Dolichos biflorus* lectin. FEBS (Fed. Eur. Biochem. Soc.) Lett. 82:39-41.
15. Galun, M., Braun, A., Frensdorff, A., and Galun, E. 1976. Hyphal walls of isolated lichen fungi. Arch. Microbiol. 108:9-16.
16. Gibson, D., and Etzler, M. E. 1979. Effect of fungal infection and wounding on inactive form of lectin in *Dolichos biflorus*. Plant Physiol. Suppl. 63:134.
17. Goldstein, I. J., and Hays, C. E. 1978. The lectins: carbohydrate-binding proteins of plants and animals. Adv. Carbohydr. Chem. Biochem. 35:127-340.
18. Hamblin, J., and Kent, S. P. 1973. Possible role of phytohaemagglutinin in *Phaseolus vulgaris* L. Nature New Biol. 245:28-30.
19. Hammarström, S., Murphy, L. A., Goldstein, I. J., and Etzler, M. E. 1977. Carbohydrate binding specificity of four N-acetyl-D-galactosamine-specific lectins: *Helix pomatia* A hemagglutinin, soybean agglutinin, lima bean lectin, and *Dolichos biflorus* lectin. Biochemistry 16:2750-2755.
20. Howard, I. K., Sage, H. J., and Horton, C. B. 1972. Studies on the appearance and location of hemagglutinins from a common lentil during the life cycle of the plant. Arch. Biochem. Biophys. 149:323-326.
21. Kauss, H., and Glaser, C. 1974. Carbohydrate-binding proteins from plant cell walls and their possible involvement in extension growth. FEBS (Fed. Eur. Biochem. Soc.) Lett. 45:304-307.
22. Liener, I. E. 1976. Phytohemagglutinins (phytolectins). Annu. Rev. Plant Physiol. 27:291-319.
23. Lis, H., Sela, B.-A., Sachs, L., and Sharon, N. 1970. Specific inhibition by N-acetyl-D-galactosamine of the interaction between soybean agglutinin and animal cell surfaces. Biochim. Biophys. Acta 211:582-585.
24. Lis, H., and Sharon, N. 1977. Lectins: Their chemistry and application to immunology. Pages 429-529 in: M. Sela, ed. The Antigens. Vol. 4. Academic Press, New York.
25. Lotan, R., and Sharon, N. 1973. The fluorescence of wheat germ agglutinin and of its complexes with saccharides. Biochem. Biophys. Res. Commun. 55:1340-1346.
26. Mialonier, G., Privat, J. -P., Monsigny, M., Kahlem, G., and Durand R. 1973. Isolement, propriétiés physico-chimiques et localisation in vivo d'une phytohémagglutine (lectine) de *Phaseolus vulgaris* L. (var. Rouge). Physiol. Vég. 11:519-537.
27. Mirelman, D., Galun, E., Sharon, N., and Lotan, R. 1975. Inhibition of fungal growth by wheat germ agglutinin. Nature 256:414-416.
28. Pereira, M. E. A., Kabat, E. A., Lotan, R., and Sharon, N. 1976. Immunochemical studies on the specificity of the peanut (*Arachis hypogaea*) agglutinin. Carbohydr. Res. 51:107-118.
29. Pereira, M. E. A., Kabat, E. A., and Sharon, N. 1974. Immunochemical studies on the specificity of soybean agglutinin. Carbohydr. Res. 37:89-102.
30. Rouge, P. 1974. Étude de la phytohémagglutinéne des graines de Lentille au cours de la germination et des premiers stades du développement de la plante. Evolution dans les cotylédons. C. R. Acad. Sci. Paris 278:449-452.
31. Rouge, P. 1976. Biosynthése des hémagglutinines au cours de la maturation des graines de Pois. C.R. Acad. Sci. Paris 282:621-623.
32. Talbot, C. F., and Etzler, M. E. 1978. Development and distribution of *Dolichos biflorus* lectin as measured by radioimmunoassay. Plant Physiol. 61:847-850.
33. Talbot, C. F., and Etzler, M. E. 1978. Isolation and characterization of a protein from leaves and stems of *Dolichos biflorus* that cross reacts with antibodies to the seed lectin. Biochemistry 17:1474-1479.