J. Biosci., Vol. 5, *Supplement* 1, December 1983, pp. 1–7 © Printed in India.

Distribution and properties of a 'lectin-like' glycoprotein from leaves and stems of *Dolichos biflorus*: Implications on the role of lectins in plants

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Abstract. Studies on the distribution of the *Dolichos biflorus* lectin during the life cycle of the plant have shown that the seed lectin appears at about 27 days after flowering and is localized in the cotyledons. Although this lectin disappears during the absorption of the cotyledons, a related molecule that cross reacts with antibodies to the seed lectin, appears in the stems and leaves of the plant. Structural studies on this cross reactive material show that it appears to have one subunit in common with the active subunit of the seed lectin and another subunit that has a higher molecular weight than the seed lectin subunits. The subunits of the seed lectin and cross reactive material may all represent different degrees of completion or modification of a common gene product.

The cross reactive material has been found to have carbohydrate binding activity at low ionic strength and a significant amount of this "lectin-like" glycoprotein appears to be associated with the cell wall. Further studies on the distribution and properties of lectins during the life-cycle of the plant may be valuable in obtaining a better insight into the physiological role(s) of these molecules in the plants.

Keywords. Lectin; distribution; structure; role.

Introduction

The abundance of plant lectins and their abilities to specifically recognize particular carbohydrate residues have made them valuable tools for the characterization of complex carbohydrates and cell surfaces. However, despite the vast literature on these molecules and their variety of applications, little is known about their physiological function(s) in the plant.

A variety of roles have been proposed for plant lectins, ranging from their involvement in plant growth by serving as storage or transport proteins (Boyd, 1963), mitogenic stimulators of plant embryonic cells (Howard *et al*., 1972) or involvement in cell wall extension (Kauss and Glaser, 1974) to their use as protective agents (Boyd, 1963) or as attractants for specific strains of *Rhizobia* needed for nitrogen fixation in leguminous plants (Hamblin and Kent, 1973). Although some of these proposed roles have attracted considerable attention in recent years, there is little evidence to confirm any of these roles as a physiological function of lectins. Before such a role can be established it is essential to have more information on the distribution and properties of lectins during the life cycle of the plant. The present paper reviews the studies we have conducted on the distribution and properties of the *Dolichos biflorus* lectins and discusses the possible implications of these studies on the role(s) of lectins in plants.

Properties of *Dolichos biflorus* seed lectin

The seeds of the *Dolichos biflorus* plant contain a lectin that selectively agglutinates blood group A substance (Boyd and Shapleigh, 1954) due to its specificity for terminal nonreducing α-N-acetyl-D-galactosamine residues (Etzler and Kabat, 1970; Hammarstrom *et al.*,1977). This lectin is a tetrameric glycoprotein with a molecular weight of approximately 110,000 (Carter and Etzler, 1975a) and consists of apparently equal amounts of two types of subunits, I and II (Carter and Etzler, 1975b). These subunits have similar amino acid and carbohydrate compositions (Carter and Etzler, 1975b), identical NH_2-terminal amino acid sequences (Etzler *et al*., 1977), and show reactions of identity when run in immunodiffusion against antisera made to either subunit or to the intact lectin (Carter and Etzler, 1975b). The NH_2–terminal halves of these subunits isolated after CNBr cleavage appear to be identical (Carter and Etzler, 1975c), but the subunits differ at their COOH-terminal ends (Carter and Etzler 1975b, c) and Subunit I is slightly larger than Subunit II (Carter and Etzler 1975b). The above information suggests that these two subunits may represent different degrees of completion or modification of a common polypeptide chain.

Equilibrium dialysis studies on the lectin showed that it has two carbohydrate binding sites per molecule (Etzler *et al*., 1981). Haemagglutination, quantitative precipitin, and affinity electrophoresis studies of the isolated subunits showed that only Subunit I has carbohydrate binding properties and that this subunit may be primarily responsible for the carbohydrate binding properties of the lectin (Etzler *et al.*,1981). This suggestion is supported by our recent finding that a monoclonal antibody that appears to be directed against a determinant on the lectin at or near the active site, reacts only with Subunit I (Borrebaeck and Etzler, 1978). The small difference in structure at the COOH-terminal ends of these subunits may thus be important for the activity of the lectin.

Distribution of the lectin

The distribution of the lectin during the life cycle of the plant was followed using a sensitive radioimmunoassay based upon the ability of lectin to inhibit the precipitation of radiolabelled lectin by antibodies to the seed lectin (Talbot and Etzler, 1978a). The lectin was not detected in the seeds during the first 26 days after flowering but appeared on day 27 and reached a maximum level by the 28th day (figure 1). High levels of lectin were maintained in the dry seed where the lectin accounts for about 9% of the total nitrogen of the seed extract (Etzler and Kabat, 1970; Talbot and Etzler, 1978a).

The lectin of germinating seeds is confined to the cotyledons (Talbot and Etzler, 1978a) where it appears to be associated with the storage granules or protein bodies (Etzler *et al.* unpublished). As the cotyledons are absorbed, the lectin disappears at about the same rate as the other cotyledon proteins (figure 2) (Talbot and Etzler, 1978a). Although no lectin was detected in the roots, low levels of lectin were found in the stems and leaves of the plant (Talbot and Etzler, 1978a). Attempts to isolate and characterize this lectin from the stems and leaves showed that it was noridentical to the seed lectin but gave a precipitin band of only partial identity to the seed lectin when run in immunodiffusion against antisera to the

seed lectin (Talbot and Etzler, 1978a, b). The properties of this cross reactive material are described below.

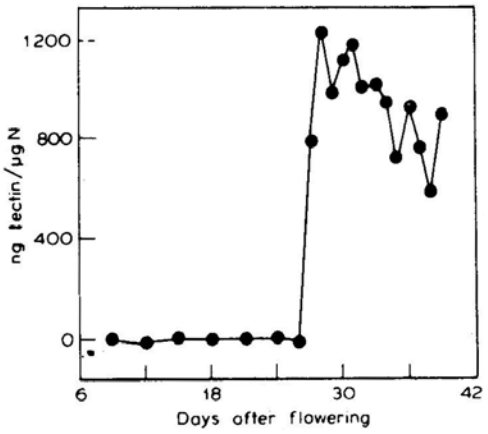

Figure 1. Lectin content of developing seeds of *D. biflorus* plant. At various time after flowering, the two to six seeds in a pod were pooled, homogenized, and assayed for lectin and nitrogen. Each point is the average of data from seeds of two to six pods except for days 29, 35, 36 and 37, when the seeds from only one pod were assayed (Reprinted from Plant Physiology **61,** 847-850, Talbot and Etzler, 1978a, American Society of Plant Physiologists).

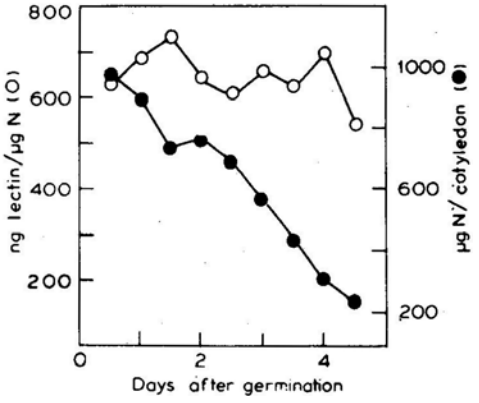

Figure 2. Lectin content of cotyledons during germination of seeds. At 12 h intervals from the time of planting, cotyledons were homogenized and assayed for nitrogen and lectin. (O), ng of lectin/µg of N; (●), total amount of N in cotyledon. Each point is the average of 12 assays. (Reprinted from Plant Physiology **61,** 847-850, Talbot and Etzler 1978a, American Society of Plant Physiologists).

Properties of 'lectin-like' glycoprotein from stems and leaves

The isolated cross reactive material from the stems and leaves of the *Dolichos biflorus* plant had a similar amino acid and carbohydrate composition to the seed lectin (Talbot and Etzler, 1978b). The cross reactive material is a dimer composed of a subunit identical in electrophoretic mobility to Subunit I of the seed lectin, and a subunit with a higher molecular weight than either of the seed lectin subunits (figure 3). Both the subunits of the cross reactive material have identical NH_2-

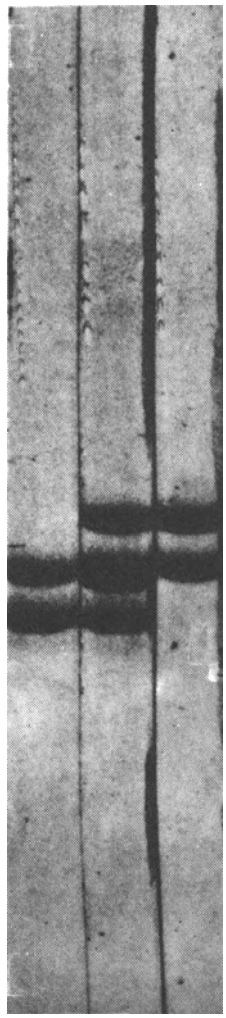

Figure 3. Discontinuous Polyacrylamide gel electrophoresis of cross reactive material and seed lectin isolated from *Dolichos biflorus* and placed on pH 9.7 glycine gels in the presence of 0.1% soidum dodecyl sulphate and 8.0M urea. The gel at left shows seed lectin subunits; the gel at right shows CRM subunits; the middle gel shows a mixture of CRM and seed lectin subunits. Gels are stained with Coomassie Brilliant Blue. (Reprinted from Phytopathology **71,** 744–746, Etzler, 1981).

terminal sequences (Talbot and Etzler, 1978b), and these sequences are identical to the seed lectin subunit sequences with the exception of an aspartate instead of an asparagine at the second residue (Etzler *et al.*, 1977; Talbot and Etzler, 1978b). This similarity of subunits indicates that the cross reactive material and lectin subunits may all be modifictions of a common polypeptide chain.

When assayed under the normal conditions for testing lectin activity, the cross, reactive material did not agglutinate nor inhibit the agglutination of type A erythrocytes nor react with blood group A substance in solution (Talbot and Etzler 1978b). Lowering the ionic strength, however, enabled the cross reactive material to bind to affinity columns of blood group A substance (Etzler and Borrebaeck,

1980). The cross reactive material may therefore be considered as a lectin in its own right. Affinity electrophoresis studies indicated that the cross reactive material may have a somewhat broader carbohydrate specificity than the seed lectin (Etzler and Borrebaeck, 1980).

Distribution of 'lectin-like' glycoprotein

Immunofluorescence microscopy and cell fractionation studies indicate that a substantial portion of the cross reactive material is associated with the cell walls of the plant. This cross reactive material can be released from the cell walls by treatment with cellulase and pectinase (Etzler *et al.,* 1979).

Using a radioimmunoassay with antibodies specific for the cross reactive material, the distribution of soluble and cell wall associated cross reactive material was followed during early development of the plant (Roberts and Etzler, 1980). Only low levels of cross reactive material were detected during the first two days of germination, but on the third day the cross reactive material content began to increase and showed a 20-fold increase by the fifth day (Roberts and Etzler, 1980). This cross reactive material was localized in the epicotyl and leaves and was almost equally distributed between the soluble and particulate fractions. No cross reactive material was detected in the roots and only low amounts were found in the hypo cotyl and cotyledons (Roberts and Etzler, 1980).

A fungal infection, classified as *Erysiphe pisi,* that occurred in our greenhouse caused an apparent elevation of cross reactive material levels in the leaves and stems of the plant,as detected by immunofluorescence microscopy. Other infections agents (*Botrytis cinerea* and *Pseudomonas phaseolicola*) and wounding the plants also produced a similar apparent elevation in the cross reactive material (Gibson and Etzler, 1979). These results must be considered as preliminary, however, since they could not be confirmed by radioimmunoassays of extracts of the infected or wounded tissues. Investigations are now in progress to determine whether this failure may be due to a degradation of the elevated cross reactive material during extraction.

Implications of these studies on the role(s) of lectins in plants

The localization of the *Dolichos biflorus* seed lectin in the cotyledons of the plant and its rate of disappearance relative to the other cotyledon proteins, suggests that this lectin may indeed play a role as a storage protein for use as food for the plant during germination as suggested many years ago by Boyd (1963). Seed lectins in some other plants have been found to have similar distributions (Lis and Sharon, 1981).

The failure to find lectin in the roots of the *Dolichos biflorus* plant does not support the hypothesis that lectins may be involved in the attraction of symbiotic *Rhizobia.* Some other investigators have also failed to find appreciable levels of lectins in roots, although other studies indicate their presence in the roots of some plants (Lis and Sharon, 1981).

The discovery of the cross reactive material in the stems and leaves of the *Dolichos biflorus* plant and its close structural relationship to the seed lectin, raise the

possibility that this molecule may be the functional lectin in the plant and that the seed lectin may be its degradation product. The ability to activate or inactivate the cross reactive material depending upon the ionic strength of the solution suggests that the activity of this lectin may be under some type of regulatory control. The association of the cross reactive material, with the cell walls of the leaves and stems and its increase during the period of rapid growth, support the suggestion by Kauss and Glaser (1974) that lectins may be involved in cell wall elongation. The preliminary indications, that wounding or fungal infection may increase the level of cross reactive material,indicate that perhaps this molecule might also function in maintaining the integrity of the cell wall in response to stress (Etzler, 1981).

A cross reactive material to a seed lectin has also been reported in the leaves of *Phaseolus vulgaris* (Mialonier *et al.*,1973) and further studies on the distribution of lectins in other plants may reveal similar 'lectin-like' substances in stems and leaves. Further studies of the distribution and properties of these molecules and their relationship to the seed lectin may aid in obtaining an eventual understanding of the role(s) of lectins in plants.

Conclusions

A study of the distribution of the *Dolichos biflorus* lectin during the life cycle of the plant showed that the seed lectin appears at about 27 days after flowering and is concentrated in the cotyledons. Although this lectin disappears during the absorption of the cotyledons, a related molecule that cross reacts with antibodies to the seed lectin begins to accumulate in the stems and leaves of the plant. This cross reactive material appears to have one subunit in common with the seed lectin and another subunit that has a higher molecular weight than the seed lectin subunits. Both the cross reactive material and seed lectin subunits may represent different degrees of completion or modification of a common polypeptide chain.

The carbohydrate binding activity of the cross reactive material can be altered by the ionic strength of the solution and a substantial portion of this 'lectin-like' glycoprotein appears to be associated with the cell wall. There are preliminary indications that the level of this molecule may be elevated upon wounding or fungal infection of the plant.

Further studies on the distribution and properties of lectins during the life cycle of the plant may be valuable in obtaining a better insight into the physiological role(s) of these molecules in the plants.

Acknowledgements

The research reported in this paper was supported by U.S. Public Health Service Grant GM21 882 from the National Institutes of Health and U.S. Department of Agriculture Grant SEA 5901-0-0242.

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