Development and Distribution of a Lectin from the Stems and Leaves of Dolichos biflorus

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ABSTRACT

The stems and leaves of the Dolichos biflorus plant contain a lectin that cross-reacts with antiserum against the seed lectin. This cross-reactive material (CRM) was followed during early seedling growth, stem elongation, and seed development using a specific radioimmunoassay.

No CRM was detected in developing seeds, but very low levels were found in dormant and imbibed seeds. As germination proceeds, the CRM accumulates at the apex of both etiolated and green seedlings in the epicotyl and leaves. Lower amounts of CRM are found in the cotyledons and hypocotyl, but no CRM was detected in the roots.

The amount of CRM in the first and second stem internodes increases during elongation and gradually declines after the completion of elongation. Approximately 80% of the CRM in the stems of 19-day-old Dolichos biflorus plants is associated with the elongating tissues. These results are discussed with respect to the possible roles of lectins in plants.

Many plants contain carbohydrate binding proteins and glycoproteins known as lectins (12, 20). Although the structural properties and carbohydrate specificities of a number of plant lectins are well characterized, little is known about their physiological function. Among the biological roles proposed for plant lectins are carbohydrate transport (4, 18), stimulation of cell division (16), cell wall extension (17), storage of seed protein reserves (4), or the packaging or mobilization of these storage materials (32), specific attractants for Rhizobial symbiosis (2, 13) and protection against plant pathogens (1, 4, 27, 28). The evidence for and against these various roles has recently been reviewed (6). Before any of these roles can be established, it is necessary to have information about the development and distribution of lectins in the plant.

The seeds of the Dolichos biflorus plant contain a lectin that is specific for terminal nonreducing α-linked N-acetylgalactosamine residues (8). This lectin appears in the cotyledons during maturation of the seeds (30), where it is primarily localized in the protein bodies (9). A developmental study of this lectin showed that it was degraded during seed germination and early seedling growth at about the same rate as the other cotyledon storage proteins (30). Although the seed lectin was not detected in other parts of the plant, a glycoprotein that cross-reacts with antibodies to the seed lectin was found in the stems and leaves (30, 31). This CRM was purified and found to contain two subunits with identical amino terminal amino acid sequences (30); these sequences are homologous to the sequences of the two subunits of the seed lectin (10). The structural similarities between these two proteins have suggested that they may represent different posttranslational modifications of a common gene product (5).

Despite the structural similarities between the seed lectin and the CRM, the CRM does not agglutinate type A erythrocytes nor bind to blood group A substance under conventional lectin assay conditions (31). However, at low ionic strength, the CRM exhibits lectin activity and has a carbohydrate specificity somewhat broader than that of the seed lectin (7). The CRM has a different subcellular localization than the seed lectin; a substantial portion of this protein has been found to be associated with the cell wall fraction (9). The present paper presents the results of a study of the appearance and distribution of the CRM during various stages of development of the D. biflorus plant.

MATERIALS AND METHODS

CRM was isolated from stem and leaf extracts of D. biflorus plants by (NH₄)₂SO₄ precipitation followed by either ion exchange chromatography or affinity chromatography on blood group A+H substance-Sepharose as previously described (7, 31). The purified CRM was radiolabeled with Na¹²⁵I (17 Ci/mg, New England Nuclear) using the iodine monochloride procedure (7, 23).

Fig. 1. CRM content of D. biflorus plants during seed germination and early seedling growth (O) µg CRM/mg protein in light grown plants; (●) µg CRM/mg protein in etiolated plants. Day 0 represents the dried seed and day 1 represents the imbibed seed. Each point is the average of data from two or three preparations except for days 0, 2, 4, and 6 which are from a single preparation. Ten plants were used for each preparation.

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3 Abbreviations: CRM, cross-reactive material; PBS, 0.01 M phosphate buffer (pH 7.2) containing 0.15 M NaCl and 0.02% NaN₃.
Rabbit antiserum against the CRM was depleted of antibodies that cross-react with the seed lectin by chromatography on a column of *D. biflorus* seed lectin covalently coupled to Sepharose and used in a competitive CRM-specific radioimmunoassay as previously described (7).

*Dolichos biflorus* seeds (F. W. Schumacher, Sandwich, MA) were imbibed by soaking in aerated water at room temperature for 20 to 24 h. After removal of seed coats, imbibed seeds were planted in vermiculite saturated with Hoagland solution A (14). Etiolated plants were grown in light proof containers fitted with air vents. Green plants were grown in a regulated growth chamber with a day length of 18 h and a light intensity of 1800 ft-c. For studies of seed development, plants were grown under greenhouse conditions as previously described (30).

Plant samples were frozen in liquid N₂ and ground to a fine powder using a mortar and pestle. Etiolated plant samples were extracted at 4°C with PBS containing 5.75 mM phenylmethylsulfonylfluoride. Green plant samples were extracted at 4°C with 0.1 M K-phosphate (pH 7.2) containing 5.75 mM phenylmethylsulfonylfluoride, as well as 0.15 M isosorbate and 2 mM thio-glycollate to prevent the modification of the CRM by plant phenols (21, 31). The plant extracts were centrifuged at 27,000 g
for 5 min and the pellets were washed twice with fresh extraction buffer. The supernatants from extractions of green plants were dialyzed at 4°C against PBS and were assayed for CRM using the radioimmunoassay. The supernatants from extractions of etiolated plants were directly assayed for CRM. After extraction, the particulate fraction was digested with 257 units/ml pectinase (Serva, 3200 μg/mg) and 14.5 units/ml cellulase (Worthington, 2.9 μg/mg) in 50 mm sodium acetate (pH 5.5) containing 0.02% NaN₃. Digestion was carried out at 37°C for 24 h after which the digest was centrifuged at 27,000 g for 5 min and the supernatant was assayed for CRM using the radioimmunoassay.

Protein concentration was determined either by the Lowry procedure (22) or by nitrogen determination using a modified ninhydrin method (29, 30).

RESULTS
CRM Content of Germinating Seeds and Young Seedlings. Using the CRM specific radioimmunoassay it was possible to measure the amounts of CRM in seeds and young seedlings despite the high concentrations of seed lectin in the plants at these stages of development.

Dolichos biflorus seeds were imbibed in aerated water for 24 h and were grown in the presence or absence of light as described above. Beginning at day 0 (dried seed), the seedlings were removed at 24-h intervals, extracted and assayed for CRM by radioimmunoassay, and for protein by the Lowry procedure (22). Extracts of dried seeds and seeds imbibed for 1 d contained very low levels of CRM that were at the limits of detection by the radioimmunoassay (Fig. 1). In etiolated plants, CRM levels began to increase on day 3 and by day 7 were 100-fold higher than that found in the imbibed seeds. The CRM accumulated more rapidly in green plants and by 4 d had reached a level 150-fold higher than that found in the imbibed seeds (Fig. 1). After day 4, the CRM levels in the green plants decreased and by day 7 were approximately the same as the amount found in etiolated plants of the same age. The distribution of CRM between the soluble

![Diagram](https://example.com/diagram.png)

**FIG. 4.** CRM content of first internode stem segments during development. A, μg CRM/stem section (○); stem section length (■). B, μg CRM/mg protein (○); stem section length (□). Points for days 3, 4, 5, 6, 8, and 19 are averages of data from two or three preparations. Points for days 2, 7, 9, 10, 11, 13, 15, and 17 are data from single preparations. Ten to 20 plants were used for each preparation.
and particulate fractions was essentially equivalent to that obtained in extracts of mature plants (9).

To determine the distribution of CRM during seed germination and early seedling growth, the plants were dissected into cotyledons, epicotyls and leaves, hypocotyls and roots, and were extracted and assayed for CRM and protein. In both etiolated and green plants, the accumulation of CRM occurs predominantly at the apex of the plant in the epicotyl and leaves (Figs. 2 and 3). By day 5, 93% of the total CRM in etiolated plants and 86% of the total CRM in green plants were present in the epicotyl and leaves. Smaller quantities of CRM were also found in the cotyledons and hypocotyls of both etiolated and green plants (Figs. 2 and 3). No CRM was detected in the roots of the plants at any point during this study.

CRM Content of Developing Seeds. As previously described, flowering was used as a reference point for seed development (30). Starting with the 14th d after flowering, seeds were removed at 2-d intervals, extracted and assayed for CRM and protein. CRM was not detected at any point during seed development and maturation.

CRM Distribution during Stem Development. Beginning with day 2, green plants were harvested at 24-h intervals and the stem sections between the leaf nodes were dissected, extracted, and assayed for CRM and protein. The amount of CRM in the first internode (hypocotyl) and second internode (epicotyl) increases and reaches a maximum level during internode elongation (Figs. 4A and 5A). After the completion of elongation, the CRM content in the internodes declines gradually. By day 19, the concentration of CRM in the first and second internodes is 5-fold less than the maximum concentration observed during internode elongation.

In contrast to the elongating stem internodes of green plants, CRM levels fluctuate very little during hypocotyl elongation in etiolated plants (Fig. 2). The maximum amount of CRM during
DEVELOPMENT AND DISTRIBUTION OF LECTIN 883

hypocotyl elongation in green plants is 70-fold higher than that of elongating etiolated hypocotyls (Figs. 2B and 4A). The elevation of CRM levels accompanying stem elongation thus appears to be associated only with elongating stems from green plants.

To determine the relative distribution of CRM among mature and elongating stem tissue, the stem internodes of 19-d-old green *D. biflorus* plants were dissected, extracted, and assayed for CRM and protein. Of the total CRM in the stem, 80% is associated with the 4th, 5th, and 6th internodes (Fig. 6). These stem sections represent elongating tissues in the 19-d-old stem. In contrast to these results, much lower levels of CRM are found in the mature stem sections (1st, 2nd, and 3rd internodes). The proportion of CRM released by cellulase and pectinase during these studies remained constant and constituted 15 to 40% of the total extractable CRM.

DISCUSSION

The *D. biflorus* plant contains two structurally related lectins that have recently been shown to have different subcellular localizations (9). The seed lectin is present in the cotyledons where it is primarily associated with the protein bodies. This lectin appears in the seeds at about the 27th d after flowering and rapidly reaches the high levels found in the mature seeds (30). The lectin disappears during the resorption of the cotyledons and has not been detected in other parts of the plant. A structurally related protein (CRM) with lectin activity at low ionic strength (7, 31) has been found in the stems and leaves of the plant; a portion of this lectin appears to be loosely associated with the cell walls of the plant (9). The development of a sensitive CRM-specific radioimmunoassay (7) has now allowed the quantitation of the CRM without the interference of the seed lectin. We have used this assay in the present work to study the distribution of CRM during seed development and germination and early seedling growth.

CRM was not detected during the development of the seeds and only very low levels of this protein were found in the dried and imbibed seeds. The CRM begins to accumulate rapidly in 2-d-old green seedlings and by 4 d reaches levels 100 times greater than those of the imbibed seed. This accumulation of CRM occurs predominantly at the apex of the plant in the epicotyl and leaves; however, low levels of CRM were also found in the cotyledons and hypocotyl. Seedlings grown in the dark do not begin to accumulate CRM until the 3rd d of development and lag behind the green seedlings in the production of this protein. As had previously been found in the case of the seed lectin (30), no CRM was detected in the roots of the plant.

The inability to detect CRM during seed development raises the possibility that it does not serve as a precursor for the seed lectin as previously proposed (31). However, since the seed accumulates lectin rapidly during seed maturation (30), it is possible that precursor pools of CRM may not accumulate or may be transient and have evaded detection.

In contrast to the seed lectin which is degraded during early seed germination (30), the CRM accumulates primarily in the growing regions at the apex of the *D. biflorus* seedling. The finding that one of the subunits of the CRM appears to have a mol wt higher than the mol wt of either of the subunits of the seed lectin (31) makes it unlikely that the appearance of CRM represents a degradation or modification of seed lectin transported from the cotyledons.

The association of the highest levels of CRM with growing tissue resembles the finding of Mishkind *et al.* (25, 26) that the wheat germ agglutinin is primarily localized in the actively growing adventitious roots and leaf bases. These authors suggested that the wheat germ agglutinin may play a fungistatic role in the actively growing tissue; another possibility supported by the results of the present study is that the lectin may have a role in the regulation of growth as originally proposed by Howard *et al.* (16).

A companion paper (9) reports studies that indicate that a portion of the CRM is associated with the cell walls of the stems and leaves of the *D. biflorus* plant. Kauss and Glaser (17) proposed that lectins may play an active role in cell wall elongation. That proposal is compatible with the results from the present study in which the levels of CRM were found to be highest in those stem internodes undergoing elongation. The finding that the CRM levels decline in these internodes as they mature (Figs. 4 and 5) rules out the possibility that the differences in the amount of CRM simply reflect a variation in the number of cells in these internodes.

It should be noted that throughout this developmental study the distribution of the CRM between the soluble and cell wall extracts of the tissue was essentially comparable to that found in the preceding study of the mature plant (9). If the CRM were involved in cell wall extension, it might be expected that the proportion of this lectin extracted from the cell wall fraction may be greater in elongating stem tissue than in mature stem tissue. However, the chemical nature of the association of the CRM with the cell walls and the biological significance of soluble and particulate CRM pools are not yet known. It must therefore be recognized that, although the CRM may participate directly in cell wall extension, it may participate in other processes such as the transport and assimilation of cell wall polysaccharides as proposed by Kauss and Glaser (17).

Low levels of lectin have been reported in the vegetative tissues of some other legumes (for review, see Etzler [6]). In some cases, such as *Phaseolus vulgaris* (3), the lectin appears to be identical to the seed lectin. A study of the distribution of this protein in the vegetative tissue during the growth of the plant showed that it was present in the hypocotyl, epicotyl, and roots and that after 6 weeks the amount in the internodes increased with the second highest internode showing the highest level of lectin (3). No correlation was made between the lectin content of the stem and stem growth. A protein that cross-reacts with antibodies to the seed lectin has also been reported in the leaves of this plant (24).

Fig. 6. CRM distribution in the stems of 19-d-old *D. biflorus* plants. Each bar represents the average of values from three preparations using 10 to 20 plants per preparation. The range of values is shown by the lines on each bar.
reported for one of the *Griphonia simplicifolia* lectins isolated from mature leaves (19) and differences have been found between the subunit structures of lectins from the bark and sieve tube sap of *Robinia pseudoacacia* (11, 15). Differences between other vegetative tissue lectins and seed lectins have also been reported (6). In light of the results of the present study and the preceding study (9), detailed analyses of the subcellular localization and development of these vegetative lectins should be of value in eventually determining the variety of roles that lectins may play in plants.

**LITERATURE CITED**

1. **Bird GWG** 1959 Agar gel studies of blood group specific substances and precipitins of plant origin. I. The precipitin of *Dolichos biflorus*. Vox Sang 4: 307–313
3. **Borregaard CAK, B Mattiasson** 1983 Distribution of a lectin in tissues of *Phaseolus vulgaris* Physiol Plant 58: 29–32
4. **Boyd WC** 1963 The lectins: their present status. Vox Sang 8: 1–32
15. **Horesi V, C Haskovec, J Kocourek** 1978 Studies on lectins XXXVIII. Isolation and characterization of the lectin from black locust bark *Robinia pseudoacacia* Biochim Biophys Acta 532: 98–104
18. **Krupe M 1956 In Blutgruppenspezifische Pflanzliche Eiweißkorper (Phytagglutine.) Ferdinand Enke Verlag, Stuttgart, pp. 1–131
31. **Talbot CF, ME Etzler** 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