Isolation and properties of a lectin from the seeds of *Mimosa invisa* L.

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Abstract. A lectin has been purified from the seeds of *Mimosa invisa* L. by gel filtration and preparative Polyacrylamide gel electrophoresis. The purified lectin was homogeneous as judged by analytical Polyacrylamide gel electrophoresis, immunodiffusion and Immunoelctrophoresis. The apparent molecular weight is 100,000; the protein is a tetramer with two types of subunits (molecular weight 35,000 and 15,000). The lectin is a glycoprotein with approximately 21% carbohydrate and interacts with Sephadex and concanavalin A-Sepharose. It agglutinates erythrocytes non-specifically, does not agglutinate leucocytes and is not mitogenic, agglutinates Mimosa-nodulating *Rhizobium* and is a panagglutinin; the agglutination is not inhibited by several simple sugars. It is thermo-stable and has no metal ions.

Keywords. Lectin; *Mimosa invisa*; *Rhizobium*.

Introduction

Lectins are sugar binding proteins or glycoproteins of non-immune origin which agglutinate cells or precipitate glycoconjugates (Goldstein et al., 1980). Though lectins were first isolated from higher plants, they have been observed to be present in many classes of the plant and animal kingdoms, including microorganisms. Lectin research was stimulated because of the specific erythroagglutinating properties, mitogenic activity and transformed cell-agglutinating properties of lectins. Lectins are currently used as tools in elucidating membrane structure and cell transformation (Lis and Sharon, 1981). Legume lectins are implicated in recognition of specific nodulating rhizobia (Dazzo and Truchet, 1983; Bauer, 1981). It was shown that a lectin from a particular legume binds only to the corresponding rhizobial species and not to rhizobia infecting other legumes. This specific interaction has not been studied in Mimosoideae. The majority of the legume lectins studied belong to the tribe papilionoideae. This report describes the isolation and partial characterization of a *Rhizobium*-binding lectin from the seeds of *Mimosa invisa* L., a tropical legume of Mimosoideae.

Materials and methods

Seeds of *M. invisa* were collected from the Central Plantation Crops Research Institute, Hirehalli, Karnataka. Rhizobia used for agglutination studies were isolated...
in the laboratory from nodules of *M. invisa*. Sephadex gels, Standard sugars, arabinogalactan, xylan, mannan, standard lectins, marker proteins, were purchased from Sigma Chemical Co., St. Louis, Missouri, USA. Bio-gels were obtained from Bio-Rad Laboratories, Richmond, California, USA. Other chemicals used were of analytical reagent grade.

**Isolation of lectin**

Dehusked *M. invisa* seeds were finely ground and defatted by extraction with hexane (1:2, w/v) in a liquid nitrogen/isopropanol bath. The extract was filtered through Whatman No. 1 filter paper and air dried. Seed powder (1 g) was extracted by grinding in a mortar and pestle with 10 ml of 10 mM potassium phosphate buffer, pH 7·2, containing 150 mM NaCl (phosphate buffered saline, PBS), 1 mM MgSO₄, 50 mM sodium ascorbate and 0·3 g acid washed polyvinylpyrrolidone (PVP) (Dazzo *et al.*, 1978). All the steps were performed at room temperature. The extract was filtered through cheesecloth, and the filtrate centrifuged for 1 h at 27,000 *g* at 4°C. The supernatant was treated with ammonium sulphate (100% saturation) to recover total protein. The protein was further subjected to ammonium sulphate precipitation (45% saturation), and the precipitate was dissolved in PBS (10 mM potassium phosphate, pH 7·2, 150 mM NaCl) and desalted using a Sephadex G-25 column. Protein fractions were collected, concentrated by lyophilization and applied to a column of Bio–gel P-100. Fractions containing the lectin were pooled and electrophoresed through 5% Polyacrylamide slab gels. Part of the gel was stained with Coomassie brilliant blue G, and the lectin band was cut, eluted with PBS and the preparative Polyacrylamide gel electrophoresis (PAGE) step repeated till a homogeneous preparation was obtained.

**Affinity chromatography on Concanavalin A-Sepharose**

Concanavalin A (ConA)-Sepharose was prepared as described by Etten and Saini (1977). Elution of the bound lectin was tried with 0·1 M glucose, 0·1 M α-methyl-D-glucoside in 1 M NaCl, 0·1 M α-methyl-D-mannoside + 50% (v/v) ethylene glycol, acetic acid-sodium acetate buffer, pH 3·6 with 1 M NaCl and 1 mM each of CaCl₂, MnCl₂ and MgCl₂, and detergent buffer (1·4% cetyltrimethylammonium bromide/1 M NaCl in 50 mM acetic acid-sodium acetate buffer). Sugar concentrations upto 2 M did not elute the bound lectin.

**Analytical methods**

Analytical PAGE was performed according to Davis (1964). Electrophoresis was done using 7.5% gels at pH 8·6 for 4 h at a current density of 4 mA per tube (65 × 6 mm). Gels were stained with Coomassie brilliant blue R and destained with 7% (v/v) acetic acid in 50% (v/v) methanol. PAGE in the presence of sodium dodecyl sulphate (SDS) was performed as described by Laemmli (1970) in 8% gels. The approximate subunit molecular weight (*M*ₚ) values were obtained from the electrophoretic mobilities by comparison with those of cytochrome *C* (*M*ₚ 13,000), ovalbumin (*M*ₚ 45,000) and bovine serum albumin (BSA) (*M*ₚ 68,000). *M*ₚ