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Purification of lectins from the stems of peanut plants

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The stem of the peanut plant contains two lectins, a methyl α-mannoside specific lectin (SL-I) and a lactose/cellobiose specific lectin (SL-II). These lectins are found to be developmentally regulated and maximum activities are observed in 3–4-weeks-old plants. The two lectins SL-I and SL-II have been purified from 3-week-old stem by affinity chromatography on Sephadex G-50 and guar gum matrices respectively. Both are glycosylated lectins and have the identical subunit molecular weight of 31 kDa.

Keywords: Stem lectin; peanut

Introduction

Lectins are most abundant in the seeds of various plants, especially those of the legumes. However, they are not confined to seeds, but are often found in the vegetative parts of the plants [1, 2]. The presence of lectins in various parts of the plant and the change in their concentration during plant growth and development are important and may give clues to the biological function of the lectins. Some legumes are now reported to have more than one vegetative tissue lectin and these are not always identical to their seed counterpart and are differentially distributed in the plant tissues [1, 3–6]. An excellent example of such a legume is peanut (*A. hypogaea*) which is under investigation in our laboratory. T-Disaccharide specific lectin (PNA) is abundant in peanut seeds. Our laboratory has previously characterized two lectins PRA-I and PRA-II in developing peanut roots [6–8]. Here we report the identification and purification of two different lectins SL-I and SL-II from the stem of this plant.

Materials and methods

Peanut seeds (cv JL-24) were obtained from Groundnut Research Centre, Junagarh, India. Crosslinked guar gum was a gift from Dr P. S. Appukuttan, Sree Chitra Tirunal Institute for Medical Sciences and Technology, India. Coomassie brilliant blue R-250, 2-mercaptoethanol, acrylamide, N,N′-methyl bis acrylamide, TEMED, ammonium persulphate, agarose, sialidase, protein molecular weight kits, Sephadex G-50 were purchased from Sigma Chemical Co., USA; other reagents were of analytical grade.

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Germination

Peanut seeds were first rinsed with 95% ethanol and immersed for 3 min in acidified 0.2% mercuric chloride, washed thoroughly with sterile water and germinated on moist filter paper in Petri dishes. Germinated seeds were transferred to plain agar medium in culture tubes and grown under aseptic conditions in growth chambers maintained under 16 h:8 h light:dark cycle at 28–30 °C. For developmental studies plants were grown in the field during May–June (30–38 °C).

Preparation of extract

Stem tissues were excised and homogenized at 4 °C in phosphate buffered saline (PBS). The homogenate was centrifuged at 15000 × **g** for 30 min at 4 °C to yield a clarified crude extract. An equal volume of ice-cold acetone was added to the supernatant with constant stirring, allowed to stand on ice for 30 min and centrifuged at 5000 × **g** for 30 min at 4 °C to collect the precipitate. The pellet was air dried and suspended in PBS. The insoluble material was removed by centrifugation at 5000 × **g** for 10 min at 4 °C. This supernatant was designated the 'extract'.

For the developmental studies the extracts were prepared from plants of different age groups grown in the field, whereas the purification of the lectins was carried out with the extract obtained from 3-week-old plants grown in the growth chamber.

Affinity purification of lectins

The 'extract' containing 15 mg protein was passed through a column (0.9 × 15 cm) of Sephadex G-50 which had been previously equilibrated with PBS. The column was thoroughly washed with this buffer at a flow rate of

12 ml h^{-1} until the absorbance at 280 nm became zero. The bound protein was eluted with 0.2 M glucose in PBS. Protein content in the fractions was monitored by ultraviolet absorption spectroscopy at 280 nm. The fractions containing the highest protein concentration were dialysed against PBS and assayed. This lectin was designated as SL-I.

Unbound protein (~10 mg) from the Sephadex column was loaded on to a 0.6 × 9 cm, cross-linked guar gum column equilibrated with PBS. The unabsorbed protein was removed by washing with PBS and the bound protein was eluted with 0.2 M galactose in PBS. This lectin was designated SL-II. Peanut seed lectin (PNA) and root lectin PRA II were purified as described earlier [6].

Haemagglutination assays

Lectin activity of SL-I was assayed at room temperature (25 °C) using 2% rabbit erythrocyte (R-RBC) suspension, while SL-II activity was assayed using both rabbit and human erythrocytes (H-RBC) (2%) at room temperature as described previously [9]. The reciprocal of the highest dilution of the lectin giving complete agglutination in 20 min was considered as the haemagglutination titre. One haemagglutination (HA) unit is defined as the minimum amount of protein required for 100% agglutination under the above assay conditions. Specific activity of the lectin is defined as the number of HA units per mg protein. Haemagglutination inhibition assays were carried out at room temperature using 2% erythrocyte suspension as previously described [10]. Sialidase treatment of the erythrocyte suspension (2%) was performed at 37 °C using 0.5 U enzyme ml^{-1} as previously reported [6].

Polyacrylamide gel electrophoresis

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Laemmli [11] in 10% gel with Tris glycine buffer (pH 8.5) containing 0.1% SDS (w/v) as running buffer. The purified lectins were dissolved in the sample buffer containing 2% SDS (w/v) in the presence of 2.5% 2-mercaptoethanol (v/v) and heated to 100 °C for 5 mins before loading on to the gel.

Immunochemical method

Antisera against the stem extract, purified PRA II and PNA were raised in rabbits and Ouchterlony immunodiffusion was carried out as previously described [10].

Determination of protein and neutral sugar

Protein was assayed by the method of Lowry *et al.* (12) using bovine serum albumin as standard. Carbohydrate content was determined by the orcinol-sulfuric acid method of White and Kennedy [13] using glucose as standard.

Results and discussion

The extract of the 3-week-old peanut stem agglutinated R-RBC as well as H-RBC. Rabbit erythrocyte agglutinating activity was inhibited by glucose (25 mM per 4 HA units), while both galactose (1.56 mM per 4 HA units) and lactose (0.79 mM per 4 HA units) inhibited the H-RBC agglutinating activity of the extract. Both the agglutinating activities seemed to be developmentally regulated as shown in Fig. 1. The level of R-RBC agglutinating activity was minimal in the first week and reached its maximum level by the fourth week and then declined in the following two weeks. H-RBC activity reached a high level at the first week and that level persisted until the third week and then declined sharply.

Rabbit erythrocyte agglutinating activity (SL-I) bound to Sephadex G-50 and eluted with 0.2 M glucose as a single protein peak while H-RBC agglutinating activity (SL-II) bound to guar gum matrix and was eluted with 0.2 M galactose (Fig. 2). The yield of SL-I was 170 μg per mg protein while SL-II was only 20 μg per mg total protein. Glucose inhibition of R-RBC agglutination by the extract may be caused by the presence of SL-I in a higher proportion than SL-II. It is also observed that the agglutinating activity of purified SL-II is inhibited by 50 mM glucose as shown in Table 1. Both SL-I and SL-II are glycoproteins containing 10% and 40% neutral sugars respectively. The purified proteins showed multiple bands in 7.5% native-PAGE (results not shown), but on SDS-PAGE under reducing conditions they moved as a single protein band corresponding to identical subunit molecular weights of 31 kDa (Fig. 3). Multiple bands in native-PAGE are not necessarily due to heterogeneity in the lectin preparations, but may be due to glycosylation variants [14, 15]. Purified SL-I and SL-II gave single precipitin bands

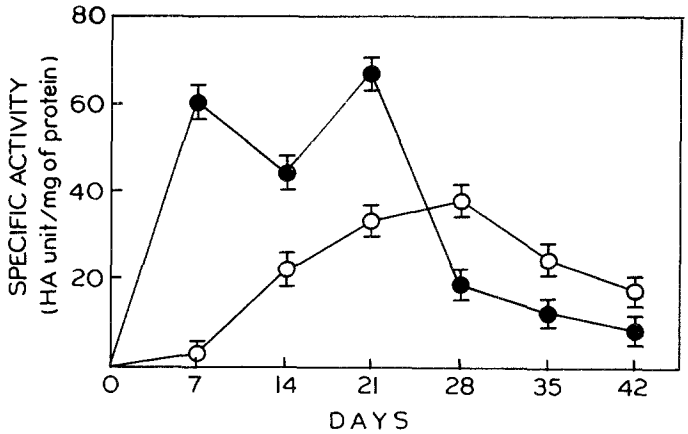

Figure 1. Variation in R-RBC (○——○) and H-RBC (●——●) agglutinating activities of stem extract prepared from 1 to 6-week-old peanut plants. Day 0 represents the imbibed seed. Each point represents the average of values from two to three preparations using 10–20 plants per preparation.

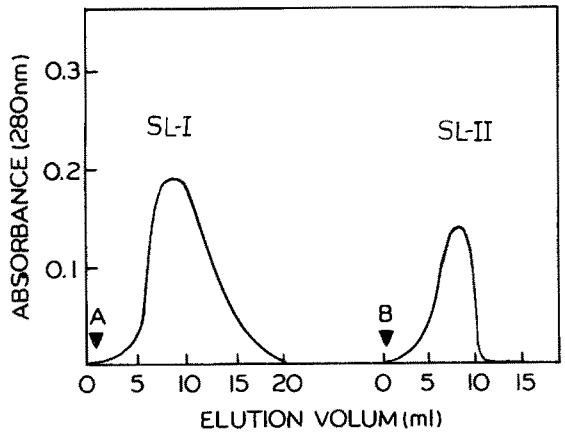

Figure 2. Sugar elution profiles of SL-I and SL-II from Sephadex G-50 and guar gum affinity matrices respectively. Stem extract was passed successively through the matrices, which were then washed with PBS until A280 was zero. Arrow A marks point of application of glucose (0.2 M) and arrow B marks the point of application of galactose (0.2 M). Experimental details are given under 'Materials and methods'.

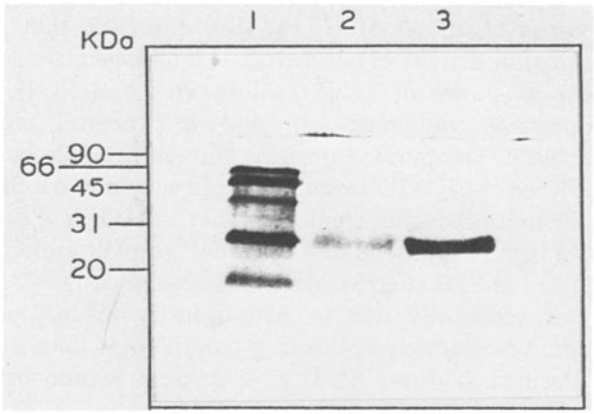

Figure 3. SDS-PAGE of purified stem lectins in the presence of 2-mercaptoethanol. Lane 1, molecular weight marker proteins (soybean trypsin inhibitor 20 kDa, carbonic anhydrase 31 kDa, ovalbumin 45 kDa, bovine serum albumin 66 kDa, transferrin 90 kDa); lane 2, purified SL-II (16 μg) lane 3, purified SL-I (60 μg).

with the antibodies raised against stem extract and the bands appeared to fuse, indicating their identical immunological cross reactivity (Fig. 4). Chelating agent, EDTA (5 mM) failed to inhibit the activities of these lectins indicating that none of them apparently have any divalent metal ion requirement. The sugar specificities of the lectins were determined by haemagglutination inhibition assays using different sugars (Table 1). Results indicate that SL-I is like other well known mannose/glucose specific lectins – viz. Con A, pea lectin, lentil lectin, etc. The most potent inhibitor is methyl α-mannoside, whereas SL-II is not inhibited by glucose, mannose or their α-linked derivatives. Purified SL-II is strongly inhibited by T-disaccharide (Gal β1-3GalNAc), lactose and cellobiose. Purified SL-I fails to

Table 1. Inhibition of haemagglutinating activity of purified peanut lectins (SL-I, SL-II and PRA-II) by different concentrations (mM)[a] of sugars.

Inhibitor	*SL-I*	*SL-II*	*PRA-II*[B]
D-Glucose	3.12	50.00	0.19
D-Mannose	3.12	NI	0.38
Methyl βD glycopyranoside	50.00	—	0.38
Methyl αD mannopyranoside	0.38	—	0.38
D-Galactose	NI	1.56	NI
Lactose	NI	0.19	NI
Cellobiose	NI	0.38	0.38
Cellotriose	NI	0.38	0.38
Cellotetraose	NI	0.38	0.38
Cellopentaose	NI	0.38	0.38
Maltose	1.56	50.00	0.76
T-disaccharide (Galβ1-3GalNAc)	—	0.09	12.5[c]
Melibiose	NI	3.12	75.00
Stachyose	—	0.78	50.00

[a] Minimal concentration required for 50% inhibition of 4 HA units of purified lectins.
[b] Kalsi *et al.* (1992) *Biochim Biophys Acta* **1177**:114–19.
[c] Non-inhibitory at indicated concentration.
NI non-inhibitory at a final concentration of 100.0 mM.
— Not tested.

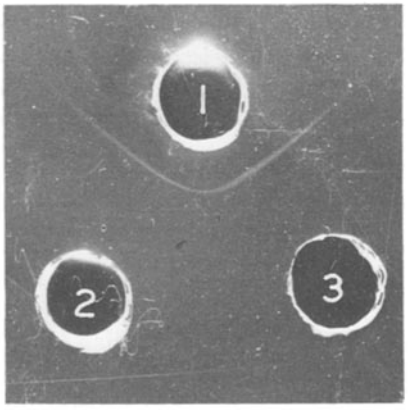

Figure 4. Immunodiffusion pattern of purified peanut stem lectins. Well 1, antiserum raised against the 'extract' of 3-week-old stem; well 2, purified SL-I (20 μg); well 3, purified SL-II (20 μg).

agglutinate H-RBC while SL-II can agglutinate both types of RBC, and R-RBC agglutination is inhibited by the same sugars that inhibit H-RBC agglutination.

These two stem lectins are distinct from peanut seed lectin (PNA) and root lectin (PRA-II). Unlike SL-II, T-disaccharide specific PNA is not inhibited by cellobiose; while glucose specific PRA-II [6] fails to distinguish between α and β linkage of glucose and SL-I is specific for α linked glucose/mannose only (Table 1).

The glucose/mannose specific haemagglutinating activity

(SL-I) resembles ML (mannose/glucose-binding lectin) detected by Law *et al.* [16] in the nodules of peanut plants.

However, SL-II is a novel lectin. So far no lectin has been reported having specificity towards lactose (Galβ1-4Glc) as well as cellobiose (Glcβ1-4Glc) and not having specificity towards glucose. T-Disaccharide is also a very potent inhibitor of this lectin.

Of all the lectins isolated so far from the peanut system [6, 8, 16, 17], only SL-II can agglutinate native human erythrocytes, however, haemagglutination titre increases with sialidase treated H-RBC. Purified stem lectins cross react with antibodies raised against either PRA-II or PNA (data not shown) indicating that they are serologically related.

Although PNA and SL-II appear to be T-disaccharide specific lectins, their specificity difference in recognition of cellobiose reflects the variation in their fine structure.

Both stem lectins are distinct from the root lectin PRA-II, which appears to play an important role in symbiosis (communicated). Further investigations are required to determine their exact functions in the host plant.

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