Phytochemistry, Vol. 27, No. 7, pp. 1951–1954, 1988.
Printed in Great Britain.

0031-9422/88 $3.00+0.00

SINGLE STEP PURIFICATION OF A LECTIN FROM WINGED BEAN TUBERS

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(*Revised received* 28 *October* 1987)

Key Word Index—*Psophocarpus tetragonolobus*; Leguminosae; winged bean; tuber; purification; lectin; affinity chromatography; cross-linked guar gum; characterization.

Abstract—A lectin from the tubers of *Psophocarpus tetragonolobus* has been purified by affinity chromatography on cross-linked guar gum. The lectin was found to be homogeneous by PAGE at pH 4.5, SDS-PAGE and gel filtration. The purified lectin is a glycoprotein consisting of two polypeptide chains containing 8% total sugars, with a M_r of 29 000 by SDS-PAGE. The hemagglutinating activity is inhibited by D-galactose and its derivatives. *N*-Acetylgalactosamine and α-galactosides are more potent inhibitors. The lectin is non-specific to human blood groups. The purified lectin is rich in acidic and poor in S-containing amino acids.

INTRODUCTION

Lectins are cell-agglutinating and carbohydrate binding proteins occuring in many plants, particularly legumes but also in bacteria and animal tissues [1, 2]. The winged bean, *Psophocarpus tetragonolobus* (L.) DC. is a tropical legume with considerable potential for development as a high protein crop [3]. The seeds of this plant contain significant amounts of protease inhibitors [4, 5] and hemagglutinins [6–9]. In recent years, the tubers of the plant have also been shown to contain some antinutritional factors including hemagglutinins [10].

In the present publication, we report the purification and some of the properties of winged bean tuber lectin and this has been compared with the lectins from the seeds of the same plant.

RESULTS AND DISCUSSION

The results of the purification steps are summarized in Table 1. Maximum lectin activity was recovered in the 30–70% ammonium sulphate precipitate fraction. The ammonium sulphate precipitate fraction yielded two protein peaks (Fig. 1) on a guar gum column. The first prominent protein peak was the unbound fraction which is devoid of lectin activity; the second protein peak due to lectin, was eluted with galactose, a specific monosaccharide inhibitor of the lectin. For the purification of lectin from winged bean tubers, cross-linked guar gum has been used, as it is a galactomannan composed of a chain of β-(1→4) linked D-mannopyranosyl residues having side stubs of single α-D-galactopyranosyl residues linked α-(1→6) to *ca* one half of the β-D-mannosyl residues. Moreover, the hemagglutination activity of the lectin was inhibited by D-galactose and its derivatives; hence the above matrix has been successfully used. A 10-fold purification of the lectin from winged bean tubers was achieved in a single step.

The homogeneity of the purified lectin preparation was confirmed by several criteria. The lectin active fraction was homogeneous by PAGE at pH 4.5 and SDS-polyacrylamide gel electrophoresis (SDS-PAGE), which gave a single band (Fig. 2). A very faint minor band was observed below the major protein band on SDS-PAGE, which may be due to the microheterogeneity of the lectin sample or an artefact. The homogeneity of the purified lectin was also confirmed by gel filtration on a Biogel P-100 column, which gave a single symmetrical protein/agglutinin activity peak.

The M_r of the purified lectin preparation was found to be 29 000 ± 1000 as determined by SDS-PAGE (Fig. 3). However, SDS-PAGE in the presence of 2-mercaptoethanol gave two closely overlapping protein bands with an M_r of 14 000 and 15 000 (Fig. 2c), which clearly implies that the lectin consists of two polypeptide chains linked together by -S-S- bonds.

The hapten-inhibition study of the winged bean tuber lectin (Table 2), revealed that it is D-galactose specific; *N*-acetyl-D-galactosamine, *o*- and *p*- nitrophenyl-α-galactosides and methyl-α-galactoside are more specific inhibitors. β-Galactosides are poor inhibitors for the lectin. Comparison of methyl-α-galactoside with methyl-β-galactoside and some of the di- and tri-saccharides containing nonreducing α- and β-D-galactopyranosyl end units reveals that the α-anomer is a much better inhibitor than the β-anomer. The binding propensities of methyl-α-galactoside, methyl-β-galactoside and galactose led to the conclusion that a methoxy group in the α-position at C-1 makes a positive contribution to the stabilization of the lectin-sugar complex. The replacement of hydrophobic moieties in D-galactose increases the binding potency, probably due to the presence of a hydrophobic region near the binding site of the lectin. The purified winged bean tuber lectin is non-specific towards any of the human blood groups tested. The purified lectin was found to be a glycoprotein as confirmed by periodic acid-Schiff's (PAS)-staining and contained 8% total sugars. The amino acid composition of the lectin (Table 3) shows

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Table 1. Summary of purification lectin from winged bean tubers

	Total protein (mg)	Total hemagglutinating activity (H.U)	Specific activity (HU/mg)	Purification (fold)	Recovery (%)
Crude extract	625	100913.0	161.0	1.0	100
Ammonium sulphate (30–70% satn)	384	96768.0	252.0	1.6	96
Affinity chromatography on cross-linked guar gum	34	85000.0	2500.0	16.0	84

Total protein was calculated for 10 gm of fresh winged bean tubers.

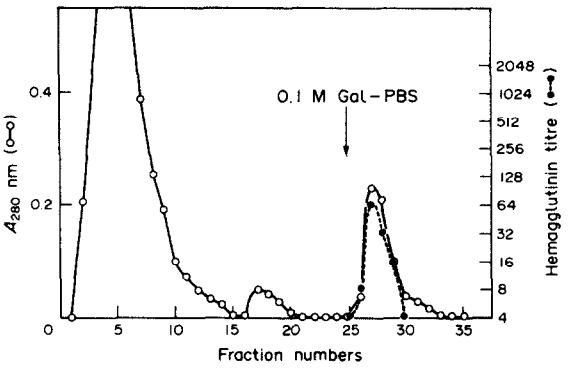

Fig. 1. Column chromatography on cross-linked guar gum of the winged bean tuber lectin. The fraction precipitating between 30–70% ammonium sulphate saturation was dialysed against 0.01 M phosphate buffer, pH 7.2 containing 0.15 M NaCl (PBS) and loaded onto the column previously developed with PBS. The adsorbed lectin fraction was eluted with 0.1 M galactose in PBS. The individual fractions were dialysed and assayed for lectin activity using trypsinized rabbit erythrocytes. (○——○) Absorbance at 280 nm; (●——●) hemagglutinin activity.

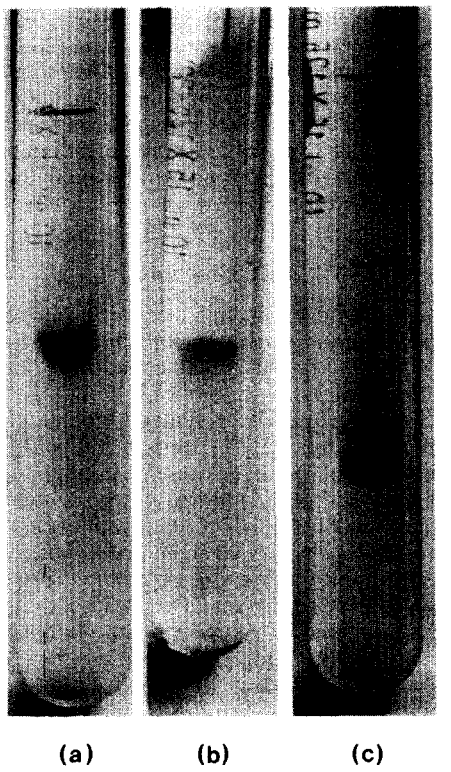

Fig. 2. Polyacrylamide gel electrophoretic pattern of the purified winged bean tuber lectin. (a) Electrophoresis according to ref. [14], using 7.5% acrylamide gel at pH 4.5; (b) SDS-polyacrylamide gel electrophoresis according to ref. [15], using 10% acrylamide gels and (c) SDS-PAGE in the presence of 2-mercaptoethanol.

fairly high amounts of glycine and acidic amino acids but only trace amounts of S-containing acids. This composition is in good agreement with other legume lectins. The M_r and some of the physico-chemical properties of the lectin were found to be very similar to the basic lectin [8, 11, 12], isolated from the seeds of same plant. Both the lectins resemble each other in their M_r by SDS-PAGE, glycoprotein nature, carbohydrate-binding specificity which is directed towards α-galactosides and amino acid composition.

The immunodiffusion pattern of the purified lectin against the crude extract of the tubers and the winged bean seeds is shown in Fig. 4. The purified lectin cross-reacted with the winged bean seed lectin(s) giving a precipitin band which indicates the immunological identity of the winged bean tuber lectin with the seed lectin from the same plant. From the above observations it appears that the two major storage tissues of winged bean synthesize essentially similar type of lectins.

EXPERIMENTAL

Materials. Winged bean tubers were collected from plants grown in Dharwad. Cross-linked guar gum was kindly supplied by Dr Salimath (CFTRI, Mysore); the SDS-M_r kit, trypsin and different sugars used for the hemagglutination inhibition study were from Sigma. Biogel P-100 (100–200 mesh) was from Biorad Laboratories. All other chemicals used were of the highest purity.

Purification of lectin. Fresh tubers were washed, peeled, cut into small pieces and homogenized with 0.15 M NaCl in a Wareing blender. The homogenate was filtered through cheesecloth and centrifuged at 4°. To the supernatant, solid $(NH_4)_2SO_4$ was added and the ppt. obtained at 30–70% satn dissolved and dialysed extensively against 0.01 M Na-Pi buffer,

Table 2. Hapten inhibition study of purified winged bean tuber lectin

Hapten	Minimal inhibitory concentration (mM)
D-Arabinose	90
D-Galactose	50
Lactose	100
Melibiose	30
Raffinose	40
Stachyose	30
N-Acetyl galactosamine	4
O-Nitrophenyl-α-galactopyranoside	4
O-Nitrophenyl-β-D-galactopyranoside	8
p-Nitrophenyl-α-D-galactopyranoside	5
p-Nitrophenyl-β-D-galactopyranoside	10
Methyl-α-D-galactopyranoside	4
Methyl-β-D-galactopyranoside	70

Glucose, mannose, fructose, L-galactose, fucose, xylose, ribose, sucrose, rhamnose, cellobiose, *N*-acetyl glucosamine, lactulose, were not inhibitory at 100 mM concentration. All sugars used were of the D-configuration unless otherwise stated.

Table 3. Amino acid composition of purified winged bean tuber lectin

Amino acid	Residues/mol	Nearest integer
Aspartic acid	12.7	13
Threonine	6.8	7
Serine	12.6	13
Glutamic acid	10.1	10
Proline	Tr*	—
Glycine	30.3	30
Alanine	13.0	13
Valine	17.1	17
Half-Cystine	nd†	—
Methionine	Tr	—
Isoleucine	7.8	8
Leucine	11.0	11
Tyrosine	19.6	20
Phenylalanine	15.0	15
Lysine	5.4	5
Histidine	4.6	5
Arginine	13.4	13
Tryptophan	nd	—

*Trace amounts
†Not determined

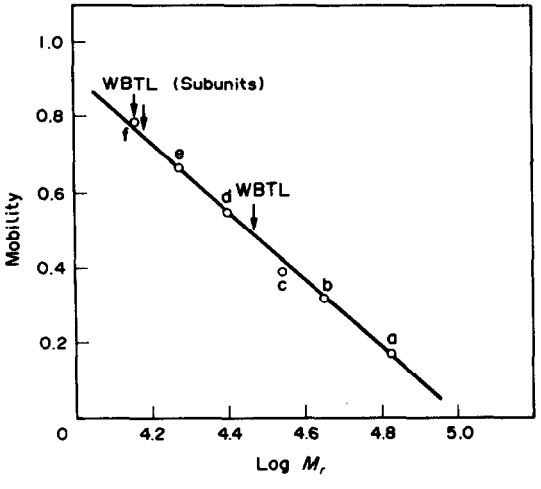

Fig. 3. Determination of M_r of ringed beam lectin by SDS-PAGE. Standard proteins: (a) bovine serum albumin (M_r 66000); (b) ovalbumin (M_r 45000); (c) pepsin (M_r 34700); (d) trypsinogen (M_r 24000); (e) β-lactoglobulin (M_r 18400) and (f) lyzyme (M_r 14300).

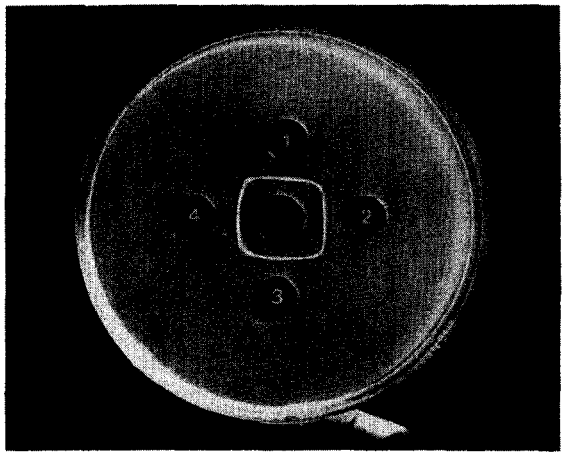

Fig. 4. Immunodiffusion pattern of purified lectin. 1% agar in PBS was used. Antibodies were placed in the centre well. (1 and 3) purified lètin; (2 and 4) crude extracts of tubers and seeds of winged bean, respectively.

pH 7.2, containing 0.15 M NaCl (PBS) in the cold. The clear supernatant obtained after centrifugation of the dialysate was applied to the cross-linked guar gum column. The column (1.5 × 12 cm) was developed with PBS and the bound lectin fraction desorbed with 0.1 M galactose in the starting buffer. All column operations were carried out at 4–5° unless otherwise stated.

Hemagglutinating assay. This was assayed using trypsinized rabbit erythrocytes by the serial two-fold dilution method [13]. The hapten-inhibition study of the purified lectin was carried out by mixing different concentrations of the inhibitor with four hemagglutinating units of the lectin before addition of erythrocytes and determining the lowest concn giving complete inhibition of agglutination.

Polyacrylamide gel electrophoresis. PAGE was carried out at pH 4.5 according to ref. [14] using 7.5% gels. PAGE in presence of SDS was performed by the method of ref. [15] using 10% gels. After electrophoresis, gels were stained with Coomassie Brilliant Blue R-250 for protein and also with periodic acid–Schiff reagent for glycoprotein [16].

Protein and carbohydrate determination. Protein content was determined according to ref. [17] using BSA as standard. Total sugar content of the purified lectin preparation was determined

by the phenol–H_2SO_4 method [18] using D-glucose as ref. sugar.

Amino acid analysis. The purified lectin (1 mg) was hydrolysed in an evacuated sealed tube, with 6 N HCl for 24 hr at 110°. The hydrolysate was analysed using an amino acid analyser linked to a recording integrator.

Immunological study. In order to compare the immunological cross-reactivity of the isolated lectin with crude exts of the tubers and the lectin (s) present in the seeds of the same plant, antibodies were raised in rabbits against the purified tuber lectin. Immunodiffusion was carried out using 1% agar in 0.05 M Pi buffer, pH 7.2 containing 0.15 M NaCl [19].

Acknowledgements—M. S. S. gratefully acknowledges the financial assistance given by the University Grants Commission, New Delhi for the award of a Senior Research Fellowship.

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