Phytochemistry, Vol. 28, No. 11, pp. 2897–2900, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00

PURIFICATION AND PARTIAL CHARACTERIZATION OF A LECTIN FROM *ARIOPSIS PELTATA* TUBERS

SHRIKANTH P. HEGDE, MANJUNATH S. SHET and M. MADAIAH*

Biochemistry Division, Department of Chemistry, Karnatak University, Dharwad-580 003, India

(*Received* 14 *April* 1989)

Key Word Index—*Ariopsis peltata*; Araceae; tuber; purification; lectin; characterization.

Abstract—Lectin activity has been detected in the tubers of *Ariopsis peltata*. The lectin was purified to homogeneity by conventional chromatographic methods and its purity confirmed by PAGE at both acidic and basic pHs, SDS-PAGE and gel filtration. The purified lectin is a glycoprotein containing 7.3% total sugars. Its M_r was determined to be 11 750 $\pm$ 500 and 44 500 $\pm$ 1 000 by SDS-PAGE and gel filtration respectively, which indicate the tetrameric nature of the lectin. The carbohydrate-binding specificity of the lectin was directed towards D-galactose and its oligomers. High inhibitory activity was found with the desialylated glycoproteins: α_1-acid glycoprotein and fetuin. The lectin agglutinates erythrocytes of human 'ABO' blood types equally well, but only after neuraminidase treatment. This lectin was found to be stable over a broad range of pH (6–12) and heat labile.

INTRODUCTION

Lectins are a class of multivalent carbohydrate-binding proteins which are non-immune in origin and are assayed as haemagglutinins [1]. They are widely distributed in nature and have been isolated from microorganisms, plants and animal tissues [2]. Hitherto, more than 150 lectins have been purified and well-characterized. They display a wide variety of biological properties and this has attracted much attention. Several hypothesis concerning their biological function have been advanced, but this remains an unsettled question [3, 4].

Ariopsis peltata is a tuberous plant which is consumed in the developing countries of the humid tropics. Recently we have reported the lectin activities in different plant tubers, rhizomes and bulbs including *A. peltata* tubers [5]. Here, we describe the isolation, purification and some of the properties of the *A. peltata* tuber lectin.

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. This fraction on chromatography on a CM-Sephadex C-50 column yielded two major and two minor protein peaks (Fig. 1). The prominent protein peak showed maximum lectin activity. The other peaks contained only small amounts of lectin activity. These findings establish that *A. peltata* tuber contains more than one lectin. Even though in most cases only one lectin from one particular tissue has been reported, *Cytisus scoparius* seeds and *Ulex europaeus* seeds were reported to contain more than one lectin, [6, 7] like *A. peltata* tubers. It seems possible that plants may contain families of lectin genes which may have altered patterns of expression in a tissue or in different tissues of the same plant. These may also differ with respect to post-translational modifications of common lectin gene products [8].

The fraction with maximum lectin activity eluted from the CM-Sephadex C-50 column was found to be electrophoretically heterogeneous and was subjected for further purification on a SP-Sephadex C-50 column. This gave two protein peaks (Fig. 2). The prominent protein peak showed maximum lectin activity. The microheterogeneity of the above active fraction was lost on chromatography

Table 1. Summary of the purification of the lectin from *A. peltata* tubers

Preparative steps	Total protein (mg)	Specific activity ($10^8 \times$ H.U./mg)	MCA† ($10^{-4} \times$ ng)	Purification (fold)	Recovery (% in terms of activity)
1) Crude extract	262	3.8	25.9	1.0	100‡
2) 30–70% $(NH_4)_2SO_4$ fraction	62	15.4	6.5	4.0	94
3) CM-Sephadex C-50 cc	24	33.9	2.9	9.0	80
4) SP-Sephadex C-50 cc	14	49.6	2.0	13.0	68
5) Biogel P-150	12	53.6	1.8	14.0	63

*Haemagglutinin unit.
†Minimum concentration of protein required for agglutination.
‡100% = 10.1×10^{10} H.U.

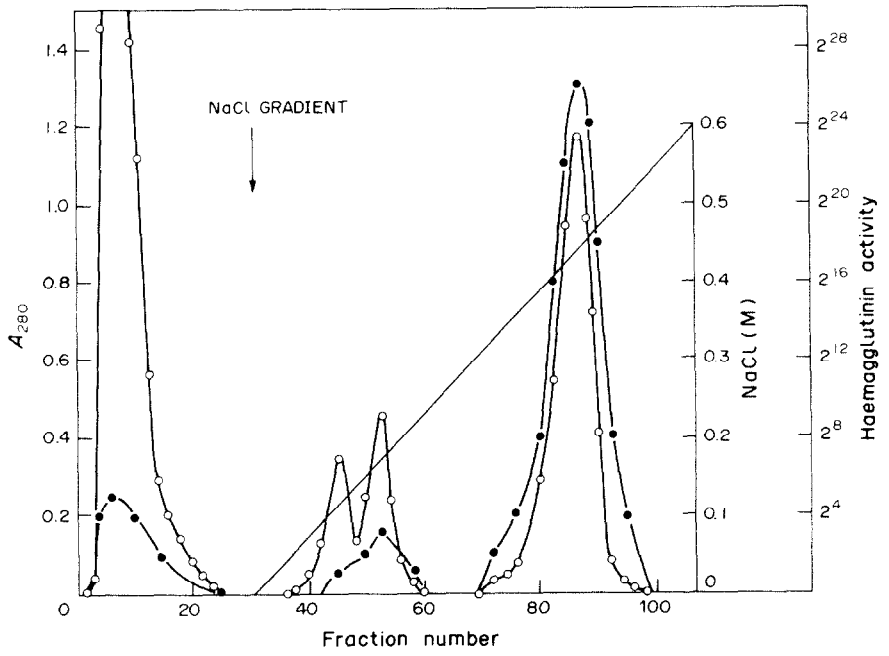

Fig. 1. CM-Sephadex C-50 column chromatography of the 30–70% ammonium sulphate fraction from *Ariopsis peltata* tubers. Column size, 2.0 × 31 cm; flow rate, 24 ml/hr; fraction size, 4.0 ml; elution buffer, 0.01 M, phosphate buffer, pH 6.0; salt gradient 0.0 to 0.6 M NaCl in elution buffer. ○—○, A_{280}; ●—●, haemagglutinin activity.

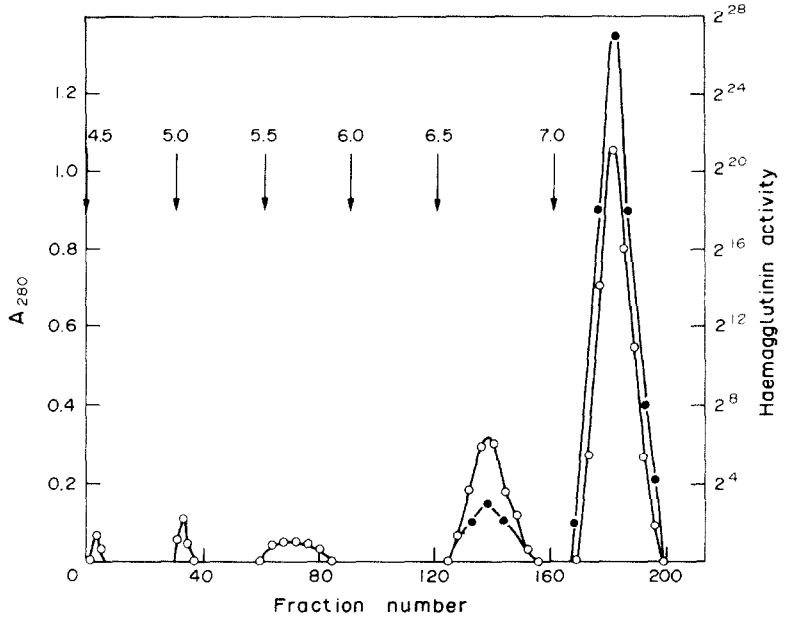

Fig. 2. SP-Sephadex C-50 column chromatography of the CM-Sephadex column eluted active fraction. Column size, 2.0 × 11.5 cm; flow rate, 30 ml/hr; fraction size, 3.0 ml; elution, stepwise pH gradient of 0.01 M acetate buffer. pH 4.5 to 7.0. ○—○, A_{280}; ●—●, haemagglutinin activity.

on Biogel P-150 column. The lectin eluted as a single symmetrical protein/agglutinin activity peak (Fig. 3) and was found to be homogeneous with 14-fold purification.

The homogeneity of the purified lectin preparation was confirmed by different criteria. The purified lectin was found to be electrophoretically homogeneous at both acidic (pH 4.5) and basic (pH 8.3) buffer systems; SDS-PAGE gave a single protein band. The homogeneity of the purified lectin was also confirmed by gel filtration on a Biogel P-150 column, which gave a single symmetrical peak.

The M_r of the purified lectin was found to be 44 500 ± 1 000 as determined by gel filtration chromatography. However, SDS-PAGE of the purified lectin in the presence and absence of 2-mercaptoethanol gave a single protein band with a M_r of 11 750 ± 500. Furthermore, gel

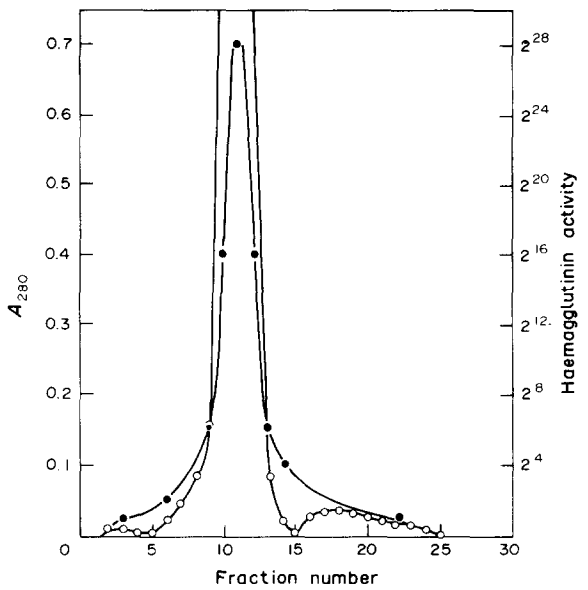

Fig. 3. Biogel P-150 column chromatography of the SP-Sephadex eluted active fraction. Column size, 1.6 × 80 cm; flow rate, 9.6 ml/hr; fraction size, 3.2 ml; elution buffer, 0.01 M phosphate buffered saline, pH 7.2. ○—○, A_{280}; ●—●, Haemagglutinin activity.

filtration of the lectin on Biogel P-150 in the presence of 0.1% SDS in the equilibration buffer (0.01 M Pi buffer, pH 7.2 containing 0.15 M NaCl) gave a single protein peak which corresponded to a M_r of 11 500 ± 500. These results establish the tetrameric nature of the *A. peltata* lectin, and indicate it to be composed of four apparently identical polypeptide chains. The purified lectin was found to be a glycoprotein as shown by periodic acid-Schiff's (PAS)-staining. The total sugar content of the purified lectin was estimated to be 7.3% as determined by the phenol–sulphuric acid method.

A hapten-inhibition study profile of the purified lectin (Table 2) revealed that haemagglutination was inhibited by D-galactose and its oligomers. The desialylated glycoproteins, fetuin and α_1-acid glycoprotein were the most potent inhibitors out of the saccharides tested. The following simple sugars did not inhibit haemagglutination even at higher concentrations: D-xylose, D-glucose, D-mannose, D-glucosamine, *N*-acetyl glucosamine, mannosamine, fucose and thiodigalactose. The lectin agglutinated rabbit erythrocytes most strongly of the erythrocytes (trypsinized and untrypsinized) of various mammalian species tested. The purified *A. peltata* lectin is non-specific to any of the human blood groups tested; moreover it did not agglutinate them. However, after treatment of cells with neuraminidase, the lectin agglutinated erythrocytes of human 'ABO' blood types equally well. Lotan *et al.* [9], have reported similar type of observation for the lectin from peanuts. The lectin was found to be stable over a wide range of pH (6–12). However, incubation of this lectin at 40° for 30 min leads to a loss of 50% of the haemagglutinin activity and 100% at 50°.

Table 2. Hapten inhibition study of purified *A. peltata* lectin

Sugars used	Minimum inhibitory concentration (mM)
D-Arabinose	50
D-Galactose	25
Lactose	100
Melibiose	100
Cellobiose	100
N-Acetyl galactosamine	50
P-Nitrophenyl-α-D-galactopyranoside	50
P-Nitrophenyl-β-D-galactopyranoside	50
O-Nitrophenyl-α-D-galactopyranoside	50
O-Nitrophenyl-β-D-galactopyranoside	50
1-*O*-Methyl-α-D-galactopyranoside	50
1-*O*-Methyl-β-D-galactopyranoside	50
α_1-Acid glycoprotein*	0.048†
Fetuin*	0.095†

*Desialylated.
†Concentration in mg/ml.

EXPERIMENTAL

Materials. Ariopsis peltata tubers were obtained from the local market in the Dharwad district. Bovine pancreatic trypsin, neuraminidase, bovine serum albumin and M_r markers used for SDS-PAGE and gel filtration were obtained from Sigma Chemical (U.S.A.). CM-Sephadex C-50 and SP-Sephadex C-50 were obtained from Pharmacia Fine Chemicals, Biogel P-150 was from Biorad Laboratories. All other chemicals used were of analytical grade of highest purity.

Purification of the lectin from the tubers. Fresh tubers (50 g) were washed thoroughly with distilled water and peeled. The peeled tuber was sliced and then homogenized in 0.01 M HCl at 4° for 5 min. The homogenate was passed through cheesecloth to remove debris and centrifuged at 10 000 rpm for 30 min in the cold. The clear supernatant was decanted and the pH was adjusted to 6.0 with 0.5 M NaOH. The ppt. formed was removed by centrifugation. The supernatant was extensively dialysed against dist. H_2O, the ppt. formed during dialysis was separated by centrifugation and the clear supernatant subjected to $(NH_4)_2SO_4$ precipitation. The ppt. fraction (30–70%) was dissolved in H_2O and dialysed extensively against dist. H_2O and used for the purification of the lectin(s).

The above fraction was loaded on to a CM-Sephadex C-50 column. The column was equilibrated with 0.01 M Pi buffer, pH 6.0, with a flow rate of 24 ml/hr. The adsorbed fractions were eluted with a linear salt gradient (0.0–0.6 M) of NaCl in the equilibration buffer. The major-lectin-positive peak was re-chromatographed on a SP-Sephadex C-50 column, previously developed with 0.01 M acetate buffer, pH 4.5. The bound fractions were eluted using a step-wise pH gradient (0.5 units/step) of 0.01 M acetate buffer of pH 4.5 to 7.0. The dominant protein/activity peak eluted with acetate buffer, pH 7.0, was purified to apparent homogeneity by this step. This fraction was once again passed through a gel filtration (Biogel P-150) column equilibrated with 0.01 M Pi buffered saline, pH 7.2 (PBS), with a flow rate of 9.6 ml/hr. All the column operations were carried out at room temp. unless otherwise stated.

Haemagglutinin assay. Haemagglutination activity of the fractions was determined by the serial two-fold dilution method as described in ref. [10] using trypsinized rabbit erythrocytes. Trypsinized erythrocyte suspension (2%) was prepared on the day of the assay according to ref. [11]. Erythrocytes from human 'ABO' blood groups were desialylated by treatment with neuraminidase as described in ref. [12]. Human erythrocytes were washed × 3 in PBS, pH 7.4, followed by centrifugation at

3 000 rpm for 5 min. A 50% packed cell suspension was treated with neuraminidase (0.7 U/ml) at 37° for 2 hr with gentle agitation every 30 min. The cells were washed × 3 with PBS and 2% suspension was used for haemagglutinin assay. Haemagglutinin inhibition study was performed using different sugars, with trypsinized rabbit erythrocytes by serially diluting the sugar solns. One unit of haemagglutinin activity was defined as the lowest concentration of lectin giving visible haemagglutination.

PAGE. Disc gel electrophoresis on polyacrylamide gel was carried out in both acidic and alkaline buffer systems according to refs [13, 14] respectively using 7.5% gels. After the run the gels were stained for protein, both with Coomassie brilliant blue R-250 and silver [15]. The gels were also stained with periodic acid Schiff's reagent to detect the glycoprotein bands, according to the method described in ref. [16]. SDS-PAGE was performed according to the procedure of ref. [17] using 10% gels, with discontinuous buffer systems.

Protein and carbohydrate content estimation. Protein content was determined by the procedure of ref. [18] using bovine serum albumin as standard. Carbohydrate content of the purified lectin was estimated by the method of ref. [19] using D-glucose as the internal standard.

M_r. determination. The M_r of the purified lectin was determined both by SDS-PAGE and gel filtration chromatography. The SDS-PAGE gels were calibrated using the following standard proteins: bovine serum albumin (M_r 66 000), ovalbumin (45 000), pepsin (34 700), α-chymotrypsinogen (25 400) and lysozyme (14 300). Gel filtration was carried out on Biogel P-150 in 0.01 M Pi buffered saline, pH 7.2, and the column calibrated with bovine serum albumin, ovalbumin, pepsin, trypsinogen and cytochrome *c*. Gel filtration on a Biogel P-150 column was also carried out in the presence of 0.1% SDS in the equilibration buffer and the column was recalibrated with the same standard proteins as described above with a flow rate of 9.6 ml/hr.

Stability of the lectin. The effect of temperature on the haemagglutinin activity of the purified lectin was determined by incubating the lectin samples at different temps. The stability of the purified lectin was checked by treating the samples at different pHs (2–12) using different buffer systems and testing for haemagglutinin activity using trypsinized rabbit erythrocytes.

REFERENCES

1. Goldstein, I. J. and Hayes, C. E. (1978) *Adv. Carbohydr. Res.* **35**, 127.
2. Gold, E. R. and Balding, P. (1976) in *Receptor-specific Proteins*. Excerpta Media, Amsterdam.
3. Sharon, N. (1979) *Glycojugate Res.* **1**, 459.
4. Lis, H. and Sharon, N. (1981) in *The Biochemistry of Plants* (Mrcus, A., ed.) Vol. 6, p. 447. Academic Press, New York.
5. Shet, M. S. and Madaiah, M. (1988) *Curr. Sci.* **57**, 1107.
6. Young, M., David, C. W. and Ross, E. W. (1984) *Biochem. J.* **222**, 41.
7. Matsumoto, I. and Osawa, T. (1969) *Biochim. Biophys, Acta* **194**, 180.
8. Marilynn, E. E. (1985) *Ann. Rev. Plant Physiol.* **36**, 209.
9. Lotan, R. L., Ehud, S., David, D. and Sharon, N. (1975) *J. Biol. Chem.* **250**, 8518.
10. Shet, M. S. and Madaiah, M. (1987) *J. Sci. Food Agric.* **41**, 338.
11. Lis, H. and Sharon, N. (1972) *Methods Enzymol.* **28**, 360.
12. Sadler, J. E., Paulson, J. C. and Hill, R. (1979) *J. Biol. Chem.* **254**, 2112.
13. Reisfeld, R. A., Lewis, U. J. and Williams, D. E. (1962) *Nature* **195**, 281.
14. Davis, B. J. (1964) *Ann. N. Y. Acad Sci.* **121**, 404.
15. Merril, C. R., Goldman, F., Sedman, S. A. and Ebert, M. H. (1981) *Science* **211**, 1437.
16. Zacharius, R. M., Zell, T. E., Morrison, J. H. and Woodlock, J. J. (1969) *Anal. Biochem.* **30**, 148.
17. Laemmli, U. K. (1970) *Nature* **227**, 680.
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. H. (1951) *J. Biol. Chem.* **193**, 265.
19. Dubois, M., Giller, K. A., Hamilton, J. K., Rebers, P. A. and Smith, P. (1956) *Anal. Chem.* **28**, 350.