Phytochemistry, Vol. 23, No. 6, pp. 1229–1232, 1984.
Printed in Great Britain

0031–9422/84 $3.00 + 0.00

ISOLATION AND CHARACTERIZATION OF A LECTIN FROM THE SEEDS OF *ERYTHRINA EDULIS*

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(*Revised received* 23 *September* 1983)

Key Word Index—*Erythrina edulis*; Leguminosae; isolation; characterization; lectin

Abstract—A galactose-specific lectin was isolated from the seeds of *Erythrina edulis*. The protein was purified by affinity chromatography of the globulin fraction on an allyl-galactoside polyacrylamide gel. The hemagglutination properties, amino acid composition, A_{280}, MW of the protein and of its subunits, carbohydrate content, electrophoretic pattern and isoelectric point were determined. Comparison of its properties with those of other *Erythrina* lectins shows that the protein is a distinct member of this group of lectins.

INTRODUCTION

Lectins are widely distributed in nature as they occur in micro-organisms, plant and animal tissues [1, 2]. They have attracted much attention due to the diversity of activities displayed by them. Several hypotheses concerning their biological function have been advanced but this remains an unsettled question [2–4]. Lectins are quite abundant in the Leguminosae especially in the Faboideae subfamily; systematic observations by Makela [5] and Boyd *et al.* [6] showed their presence in ten species of the genus *Erythrina*. Other investigators extended to 14 the number of *Erythrina* species containing lectins [7–9] but no attempts were made to characterize the proteins. In 1974 Montes de Gómez and Pérez [10] reported some properties of a lectin present in the seeds of *Erythrina edulis* Triana (ex Michelli), including its galactose binding ability. In recent years several studies have been published on the isolation and physico-chemical properties of *Erythrina* lectins [11–15]. The present work describes the isolation and characterization of the lectin from the seeds of *Erythrina edulis*.

RESULTS AND DISCUSSION

Lectin isolation and purification

The seeds of *Erythrina edulis* contain a lectin which can be purified according to the steps shown in Table 1. Control experiments showed that two extractions with 1% sodium chloride were sufficient to solubilize practically all the lectin. The addition of 1% PVP lowered two and a half and 35 times the specific titers of the saline extracts and of the globulin fraction respectively. The *E. edulis* lectin was eluted from the *O*-α-D-galactosyl polyacrylamide column as a sharp peak when 0.1 M galactose (Gal) was added (Fig. 1); the first peak (eluted with 1% NaCl without Gal) was devoid of hemagglutinating activity. An *O*-lactosyl polyacrylamide gel was equally effective for the purification of the same lectin [Pérez, G. and de Miranda, M., unpublished results]. The *O*-glycosyl polyacrylamide gels have been successfully used to purify a variety of lectins, including one from *Erythrina indica* [11]. Attempts to purify the protein using gel filtration, DEAE-cellulose [10] or Sepharose 4B were unsuccessful.

Basic PAGE of the lectin purified by affinity chromatography showed two closely migrating bands. This pattern remained after rechromatography of the protein on the *O*-galactosyl polyacrylamide support. When the lectin was analysed by acidic PAGE one band was seen. A similar behaviour depending on the PAGE conditions was observed with the *Erythrina indica* lectin [11]. The content of lectin in dry seeds is 121 mg per 100 g. This figure is very close to that reported by Horejsí *et al.* [11] for the lectin from *E. indica*. In other species of *Erythrina* the lectin

Table 1. Purification of the lectin from *Erythrina edulis*

Stage	Volume (ml)	Protein (mg/ml)	Total protein (mg)	Specific titer	Purification (fold)
1 First saline extract	800	7.4	5400	2.2	1
Second saline extract	300	2.8	852	0.7	
2. $(NH_4)_2SO_4$ ppt 0–50% fraction	70	11.3	790	5.7	2.6
3. Affinity chromatography		0.28*	—	57.1	26

*Determined on an aliquot of the peak eluted with galactose. Weight of fresh seeds: 200 g (water content: 85%). The specific titer is defined as the hemagglutination titer divided by the protein concentration (mg/ml) of the assayed solution.

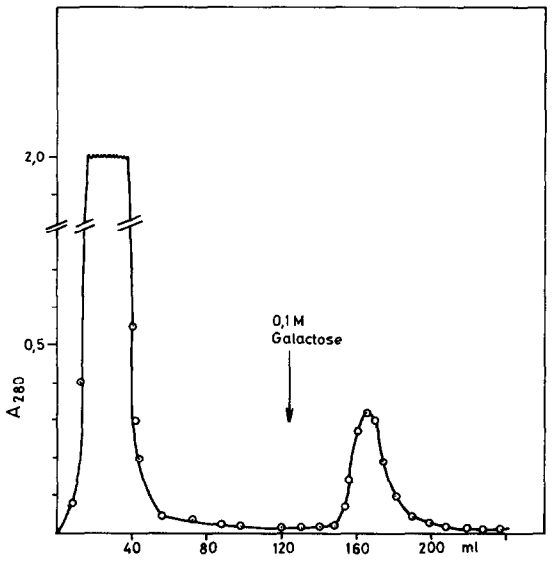

Fig 1 Affinity chromatography of the *Erythrina edulis* lectin. Elution procedure as described in the Experimental

Table 2 Carbohydrate inhibition of agglutination by the *E edulis* lectin

Carbohydrate	Minimal inhibitory concentration (mM)
D-Galactose	26 9
D-Galactosamine	105.0
N-Acetylgalactosamine	5 3
Methyl-α-D-galactoside	6 1
Methyl-β-D-galactoside	12.4
p-Nitrophenyl-β-D-galactoside	3.2
D-Lactose	1.8
D-Melibiose	14.7
D-Raffinose	54 5

The following sugars were not inhibitory at concentrations of 0 1 M: L-sorbose, D-mannitol, D-arabinose, D-xylose, D-glucose, L-fucose, D-mannose, D-fructose, D-glucosamine, *N*-acetylglucosamine, D-galacturonic acid, *N*-acetylneuraminic acid, D-sucrose, D-cellobiose, D-melezitose

content ranges from 2–180 mg per 100 g dry seeds [13, 15].

Characterization of the lectin

The lectin showed similar titers for the A, B and O blood groups. The purified protein agglutinated cow, rabbit and toad (*Bufo marinus*) red blood cells (RBCs) but not those of horse or dog. A treatment of the animal erythrocytes with trypsin caused the agglutination of all of them. There are significant differences between the lectins of the various *Erythrina* species regarding their abilities to agglutinate untreated rabbit or cow RBCs [5, 9, 12–15]. In the few cases where untreated dog or horse RBCs have been tested, a consistent lack of agglutinability has been observed and proteolysis always rendered them agglutinable [12, 13]. The agglutination activity of the purified lectin was inhibited by carbohydrates containing galactosyl residues (Table 2). Among the various carbohydrates tested, lactose was the most potent inhibitor (Minimal Inhibitory Concentration, MIC = 1.8 mM) followed by *p*-nitrophenyl-β-D-galactoside (MIC = 3.2 mM). As a whole the MIC values are similar to those determined with other *Erythrina* lectins [11, 13–15]. The displayed activity of the tested inhibitors seems to support the role of additional residues and of hydrophobic interactions in the enhancement of the inhibition as discussed by Kaladas *et al.* [16]. Galactosamine and raffinose showed the lowest inhibitory activities and their MICs were higher than those observed with other *Erythrina* lectins [13, 15]. It seems therefore that inhibition of the lectin from *E. edulis* requires the presence of C'-3, C'-4 and C'-6 hydroxyls. The presence of an acetamido group at C'-2 increases the inhibitory activity, and an amino group decreases it. The agglutination of rabbit and trypsinized horse erythrocytes was also inhibited by carbohydrates containing galactosyl residues.

The neutral sugar content of the *E edulis* lectin was 7.8 %. All *Erythrina* lectins studied so far are glycoproteins with neutral sugar contents ranging from 2.8–12 % and some possess glucosamine as the main hexosamine; the lectin from *E. edulis* showed no traces of this sugar when the appropriate zone of the amino acid analysis profile was examined.

Gel filtration of the native protein in Sephadex G-200 gave a MW of 56 000. This value is very close to MWs reported for the lectins from *E. crista-galli* [15] and *E. indica* [11]. Additional experiments using Sephacryl S-200 in identical conditions, showed some retention of the lectin and also of several protein markers. This behaviour can be explained by the increased hydrophobic character of this gel support as compared to Sephadex G-200. It is noteworthy that the apparent MW of the *E. corallodendron* lectin determined on BioGel P60 [12] is twice the average MW of the other *Erythrina* lectins studied so far [11, 13, 15, this work]; it may be then advisable to check eventual side effects due to this support. PAGE-SDS evidenced a single band with a MW of 27 000 ± 500. This figure is similar to those determined for the subunits of some *Erythrina* lectins [13, 15]; however it should be kept in mind that these values are likely to be overestimates since all these lectins are glycoproteins.

The results of the amino acid analysis are shown in Table 3. With respect to other *Erythrina* lectins [11, 13, 15] it has larger amounts of Lys and of Trp. The latter is probably the cause of the higher $A^{1\%}_{1cm}$ value (16.73) as compared to those reported for the lectins from *E. indica* [13] and *E. crista-galli* [15]. As for many other *Erythrina* lectins, sulfur amino acids were very scarce. Metal analysis of the protein showed the presence of 6–7 Ca atoms per mol of protein and of traces of Mn. Other metals such as Mg, Cu, Ni and Zn were absent. The lectin differs from those of *E. indica* [11] and *E. crista-galli* [15] in having more Ca and less Mn. Exhaustive dialysis against chelating agents did not diminish the erythroagglutinating activity. Therefore the metals are either firmly bound to the protein or they are not essential for the agglutinating activity.

The PI determination gave two protein bands which

Table 3. Amino acid composition of the *Erythrina edulis* lectin

	g AA/ 100 g protein	Calculated residues/mole	Nearest integer
Lysine	4.24	89.4	89
Histidine	0.72	14.4	14
Arginine	0.37	6.6	7
Aspartic acid	2.22	51.6	52
Threonine	1.27	33.0	33
Serine	1.21	35.4	35
Glutamic acid	2.33	49.2	49
Proline	1.34	36.0	36
Glycine	0.77	31 8	32
Alanine	0.95	33.0	33
Cysteine	—	—	—
Valine	1.25	33.0	33
Methionine	0.14	3.0	3
Isoleucine	0.84	19.8	20
Leucine	1.12	26.4	26
Tyrosine	1.05	18.0	18
Phenylalanine	0.04	0.6	1
Tryptophan*		16 7	17

*Determined colorimetrically.

Results are based on a MW of 56 000 with 7.8 % carbohydrate.

corresponded to pI values of 5.40 and 5.50. This result points to the existence of two isolectins and it is supported by the basic PAGE where two bands were also observed. A similar situation was observed by Bhattacharyya *et al.* [13] who detected three components during the pI determination of the *E. indica* lectin. Heterogeneity may well be a general feature of the lectins from the various *Erythrina* species but this point awaits further confirmation. The above results show that the lectin from *Erythrina edulis* is a dimeric glycoprotein which is able to recognize specifically galactose residues. The observed heterogeneity probably results from slight charge differences between the two subunits. The protein is a distinct member of the group of lectins from *Erythrina* species which are alike in many physicochemical properties and probably perform the same, yet unknown, function.

EXPERIMENTAL

The seeds of *Erythrina edulis* (voucher COL 38044) were collected at Sasaima, Cundinamarca

Isolation and purification. Fresh mature seeds were homogenized in a Waring blender with 1 % NaCl (1:5, w/v) and extracted at room temp. with agitation for 4–8 hr The extract was filtered through cheese-cloth and clarified by centrifugation at 3000 rpm for 20 min at 4°; the residue was extracted once more (1:2, w/v) with 1 % NaCl. After protein determination, the supernatants were pooled, made 50 % satd with $(NH_4)_2SO_4$ and kept overnight at 4°. The pptd protein was collected by centrifugation at 12 000 rpm for 20 min at 4° The protein was suspended in 1 % NaCl and extensively dialysed against the same soln. This fraction was freeze-dried and *ca* 500 mg were dissolved in 1 % NaCl and clarified by centrifugation. The soln was applied to a column (2 × 15 cm) packed with *O*-α-D-galactosyl polyacrylamide gel prepared according to ref. [17] and equilibrated with 1 % NaCl. After elution of a first peak with the equilibrating soln, 0.1 M Gal was added. The peak eluted with this soln was exhaustively dialysed against 0.5 % NaCl and freeze-dried.

Agglutination and inhibition assays Agglutination was done as described in ref [9] using human erythrocytes from slightly outdated blood. Animal erythrocytes were tested untreated and after trypsinization [9] employing purified lectin. Hemagglutination titer and sugar inhibition were determined according to ref [18].

Protein concentrations. These were estimated by the micro-Kjeldahl technique [19]. Pure lectin concns were estimated using the value $A^{1\%}_{1cm} = 16.73$ at 280 nm in 1 % NaCl. This value was obtained determining by micro-Kjeldahl the protein concn of a soln of pure lectin whose A was previously measured

Electrophoresis Basic electrophoresis was done on polyacrylamide gel slabs (11 5 × 9 cm) 3 mm thick with a stacking gel (T_3C_5, where T is the % of acrylamide plus bisacrylamide in the gel and C is the % of bis-acrylamide with respect to T) prepared in a buffer (Tris–HCl, 67 mM pH 6 7) and a separating gel (T_5C_5) in a buffer (Tris–HCl, 0.495 mM, pH 8.9). The tank buffer was Tris–Gly, 5 mM, pH 8.3. After running 2 hr at 250 V the gels were fixed for 4 hr in 10 % TCA and they were stained with Coomassie Brilliant Blue R-250 (0.025 %) in 10 % TCA. 7 % HOAc was used for destaining Electrophoresis in acidic conditions was performed in gel slabs according to ref. [20] using a $T_{7\,7}C_{2\,6}$ as separating gel

MW *determinations.* The size of the protein was estimated following the method of ref. [21]. The lectin was passed through a Sephadex G-200 column (76 × 1 cm) equilibrated with Tris–Gly, 50 mM, pH 7.5, 0.1 M KCl. A calibration curve was done with BSA, catalase, ovoalbumin, myoglobin and cytochromee (all from Sigma) The elution vols were plotted against the MWs given in ref. [22] The MWs of the subunits were determined by PAGE-SDS employing the Tris–HCl buffers and the gels described in ref [22], using a $T_{10}C_{2\,6}$ separating gel Fumarase, aldolase, α-chymotrypsinogen, trypsin and RNAse (all from Sigma) were used as reference proteins.

Neutral sugar content This was done by the method of ref. [23] using D-glucose as standard.

Amino acid analysis was carried out in a Beckman 120 C amino acid analyser. The protein was hydrolysed with 6 M HCl for 24 hr at 110° in sealed evacuated ampoules Methionine and cysteine were determined on a separated sample after performic acid oxidation [24]. Tryptophan was determined according to ref [25] after pronase hydrolysis.

Metal content. The analysis were done on a Perkin–Elmer 303 atomic absorption spectrophotometer The protein was demetallized by prolonged dialysis against 0 1 M citric acid or successive dialysis against 1 M HOAc, 0 1 M EDTA and 1 M HOAc. All solns were prepared in deionized H_2O

pI. The pI was determined on a Multiphor (LKB) apparatus according to ref. [26]. A T_5C_3 polyacrylamide gel with a 4.0–6 5 pH gradient was used The freeze-dried protein was dissolved in 0.1 M NH_4OAc and dialysed against 50 mM NH_4OAc before applying it to the gel

Acknowledgements—I am indebted to Colciencias, Organizacion de Estados Americanos and Departamento de Quimica (U Nacional) for financial support The assistance given by Dr Roberto Jaramillo (ICN, U. Nacional) is greatly appreciated.

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