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A LECTIN FROM THE SEEDS OF *ERYTHRINA RUBRINERVIA*

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Key Word Index—*Erythrina rubrinervia*, Leguminosae, isolation, characterization, lectin

Abstract—The isolation and characterization of a lectin from the seeds of *Erythrina rubrinervia* is presented. The galactose-specific lectin was purified by affinity chromatography on an allyl-galactoside polyacrylamide gel. The following properties were determined M_r, number of subunits, carbohydrate content, hemagglutination, amino acid composition, metal content and pI. Extraction of the lectin in the presence of protease inhibitors did not change its chromatographic properties, M_r or electrofocusing pattern. A comparison of its properties with those of other *Erythrina* lectins show some differences †

INTRODUCTION

Extensive botanical surveys have been conducted on the genus *Erythrina* [1, 2] mainly due to the presence of a group of alkaloids with curare-like action whose chemistry, biosynthesis and pharmacology have been studied [3] More recently, attention has been given to the nutritional value of the proteins of the edible species *E edulis* [4] and to the lectins [5–13] and the protease inhibitors present in the seeds of various species [14]. Although the majority of the *Erythrina* species are native of America (*ca* 71 out of 106) [2] only four lectins from American species have been studied [10, 11] In Colombia there are 10 recorded species for which very little information is available [2, 4, 15]. One of the species most widely distributed in Colombia is *E rubrinervia* which is also found in Bolivia, Perú and Panama [2], a few preliminary studies have been done on this species [15, 16]. As a continuation of these studies, we describe here the isolation and properties of a lectin from the seeds of *E rubrinervia* (ERL).

RESULTS AND DISCUSSION

Isolation and purification of the lectin

The high lipid content of the ground seeds (20.5%) made it necessary to defat them before extraction of the lectin. The protein was extracted and purified according to the steps shown in Table 1 Preliminary assays showed that two extractions with 1% sodium chloride solubilized 90–95% of the albumin plus globulin fractions. These extracts displayed a strong erythroagglutinating activity; a third saline extract showed faint activity. The protein was solubilized in similar amounts to those obtained from *E edulis* seeds (180 and 28 mg/g defatted seed meal, calculated from ref [10]) (Table 1). On fractionation with ammonium sulphate the lectin was completely precipitated at 50% saturation. Purification of the protein was achieved by affinity chromatography on a column packed with *O*-α-D-galactosyl polyacrylamide gel (Fig. 1) The non-retained peak was devoid of hemagglutinating activity and the lectin, eluted with 0.1 M galactose, showed the same elution volume whether or not the extraction was performed in the presence of protease inhibitors. The degree of purification of the lectin (115

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†The following abbreviated names for the *Erythrina* lectins are used: ERL, lectin from *E rubrinervia*; ECorL, *E corallodendron*, ECL, *E cristagalli*, EEL, *E. edulis*, EIL, *E indica*; ELatL, *E. latissima*, EVL, *E variegata*; EVesL, *E vespertilio*

Table 1 Purification of the lectin from *E rubrinervia*

Stage	Volume (ml)	Protein (mg/ml)	Total protein (mg)	Specific titre*	Purification (fold)	Extracted protein (mg/g flour)
1 First saline extract	1150	21 156	24330	0 76	1	173.8
2. Second saline extract	1345	3.893	5236	0 52		37 4
3. Globulin fraction	260	22 393	5965†	1 43	1 9	
4 Affinity chromatography‡	41	0 37	15 17‡	86 79	114 8	

*The specific titre is defined as the hemagglutination titre divided by the protein concentration (mg/ml) of the assayed solution

†Includes the aliquots taken for titre and N determinations on first and second saline extracts

‡15 ml of globulin fraction applied to the column

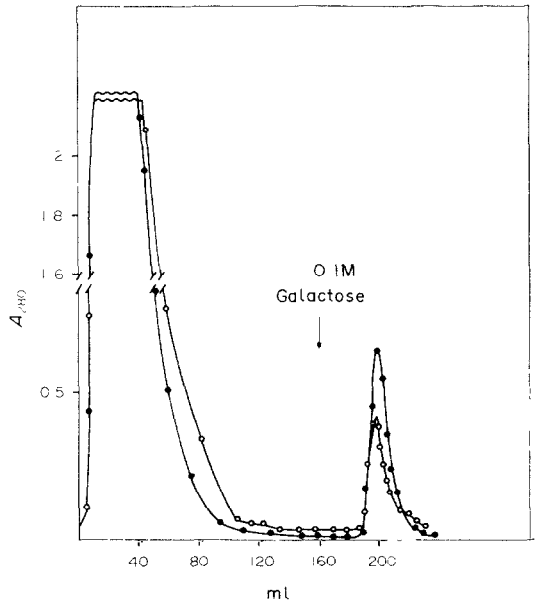

Fig 1 Affinity chromatography of the *E rubrinervia* lectin Extraction in the absence (●·· ··●) and presence (○ —— ○) of protease inhibitors Elution procedure as described in the Experimental

times, Table 1) was significantly higher than those reported for ECorL [6], EVL [8] and EEL [10]. The lectin content in dry seeds is 152 mg per 100 g This figure is within the range reported for most *Erythrina* lectins [5, 10, 11, 13]

Basic PAGE was used to monitor the different isolation steps The purified lectin appeared as two closely migrating bands, this pattern changed to a diffuse band when the protein was analysed by acidic PAGE A similar behaviour has been observed with EIL [5] and EEL [10]; it is probably due to small charge differences under basic conditions

Characterization of the lectin

Some of the molecular properties of ERL are shown in Table 2 The protein has the same gross structural features as other *Erythrina* lectins with some characteristics that deserve mention The lectin has a dimeric structure with subunits of equal M_r as opposed to several *Erythrina* lectins that by SDS-PAGE show two different subunits [5, 7, 9, 11] To check the possibility of a partial proteolysis caused by endoproteases present in the seed, such as those found in *Canavalia ensiformis* [17], we extracted the lectin in the presence of a mixture of protease inhibitors (see Experimental) The behaviour of the lectin during its extraction and purification was the same as that of the lectin in the absence of the inhibitors (Fig 1), the M_r of the lectin, determined by gel filtration, was very close (*ca* 59 000) to that previously found No differences were observed in the band patterns of the electrofocused proteins (see below) Therefore it is not likely that proteolytic cleavages have occurred during the extraction and purification of the *E rubrinervia* lectin

The carbohydrate content of ERL remains unchanged after rechromatography in 4 M guanidine to remove contaminating sugars Demetallization of the protein reduced the Ca^{2+} to 6 ions/molecule but the Mn^{2+} content was unchanged, the removal of the less tightly bound Ca^{2+} ions has no effect on the specific titer The microheterogeneity pattern observed by isoelectric focusing is also present in the protein isolated with protease inhibitors and is probably due to partial deamidation of glutamine and/or asparagine residues The hemagglutination of human blood groups A, B, O showed specific titers with similar values for all the blood groups, the minimal concentrations were 5–10 μg lectin/ml The agglutination of animal red blood cells (RBCs) is shown in Table 3 It is noteworthy that despite the close structural similarities displayed by the *Erythrina* lectins, they differ in their ability to interact with cells such as cow, dog and rabbit RBCs (for toad RBCs see ref [10]), or lymphocytes. In the latter case lectins from *E humeana E zeyheri* [11] and *E. edulis* (Moreno, C and Arango, M , unpublished results) are devoid of mitogenic activity On this basis, it is clear that similarities of physicochemical properties such as M_rs, quaternary structure, etc are not sufficient to predict any pattern of cell interaction as has been proposed recently [12]

The inhibition of hemagglutination showed that the activity was confined to D-galactose and related carbohydrates (Table 4) Maximal inhibitory activity was obtained with *p*-nitrophenyl-β-D-galactoside (Minimal Inhibitory Concentration, MIC = 6 5 mM) which is al-

Table 2 Molecular properties of *E rubrinervia* lectin (ERL)

	ERL	EIL [7]	ECL [9]	EEL [10]	ECorL [11]	ELatL [11]	EVesL [13]
M_r protein	62 000	68 200	56 800	56 000	60 246	61 980	59 000
M_r subunits	29 500	30 000	26 000	27 000	28 000	32 000	32 000
		33 000	28 000				
Bands in SDS-PAGE	1	2	2	1	1	2	1
Bands in alkaline PAGE	2	1	1	2	n d	n.d	3
% Neutral sugars	10	11 2*	2 8	7 8	5 5	9 0	9 7
Ca atoms/mol	16	2 9–3 5*	1 9	6	n d	n d	n d
Mn atoms/mol	1	1 8–2 3*	1	traces	n d	n d	n d
pI	5 19†	4 83, 5 09,	n d	5.40,	n.d	n d.	4 8,
	5 02, 5 12	5 44		5 50	n d	n d	5 3

*Data taken from ref [12]
†Main band

Table 3 Agglutination of animal erythrocytes by *E* lectins*

	Rabbit	Cow		Dog		Sheep		
	NT†	NT	T‡	NT	T	NT	T	Reference
E. rubrinervia	6–12	—	—	—	6–12	—	n d	
E. corallodendron	—	n d	n.d	—	62.5§	—	125§	[6]
E. corallodendron	5–20	n.d.	n.d.	n.d.	n.d.	—	—	[11]
E. perrieri	250–500	n.d	n.d	n.d.	n d	—	—	[11]
E indica	3 9	250	62.5	—	4 9	n.d	n d	[7]
E. cristagalli	10	n.d	n d	n d	n.d	—	—	[9]

*Data taken or calculated from references, values are expressed as minimal agglutination concentrations (μg/ml)
†Non-trypsinized erythrocytes
‡Trypsinized erythrocytes
§Papain-treated erythrocytes.

Table 4. Carbohydrate inhibition of agglutination by the *E rubrinervia* lectin

Carbohydrate	Minimal inhibitory concentration (mM)	Relative inhibitory activity				
		ERL	EEL*	ECL†	EIL‡	EVesL§
D-Galactose	53 8	1 0	1 0	1 0	1 0	1 0
N-Acetylgalactosamine	21 3	2 5	5 1	2 0	2 0	5.0
Methyl-α-D-galactoside	24 2	2 2	4 4	2.8	2 0	2.0
Methyl-β-D-galactoside	24 8	2.2	2 2	1.0	4.0	1 0
p-Nitrophenyl-β-D-galactoside	6 5	8 3	8.4	6 7	8 0	20 5
D-Galactosamine	> 105	—	0.3	0 7	0 1	0 3
D-Lactose	14.5	3 7	14.9	6 7	4.0	8 0
D-Melibiose	14 7	3 6	1.8	n d.	2 0	2 0
D-Raffinose	> 110	—	0 5	1 9	1 0	1 0

The relative inhibitory activity is calculated as the ratio: MIC of galactose/MIC of tested sugar
*Data calculated from ref [10]
†Data calculated from ref [9].
‡Data calculated from ref [7]
§Data calculated from ref [13]
The following sugars were not inhibitory at concentrations (mM) given in parentheses D-glucose (100), L-fucose (100), D-mannose (105), D-galactosamine (105), D-sucrose (120), D-cellobiose (100), D-raffinose (110), D-melezitose (80).

most 10 times as potent as D-galactose (MIC = 53.8 mM) suggesting a hydrophobic interaction similar to that studied in detail with ECL [18, 19]. The requirement of free C′-3, C′-4 and C′-6 hydroxyls in the galactosyl residue as well as the enhancing effect of an acetamido group at C′-2 seems to be a general feature of inhibitors of *Erythrina* lectins. On the other hand, there are significant differences between the *Erythrina* lectins in regard to their inhibition by some sugars; lectins from *E. indica* [7], *E cristagalli* [9], *E. edulis* [10] and *E. vespertilio* [13] are inhibited by galactosamine whereas the *E rubrinervia* lectin is not. Bhattacharyya *et al.* [7] found no galactosamine inhibition with *E lithosperma, E suberosa* and *E. arborescens* lectins. Raffinose MICs differ widely (MIC > 110 to MIC = 6.2) in a number of *Erythrina* species [7, 9, 10, this work]. The above differences suggest that the sugar-binding sites of *Erythrina* lectins from different species are similar but only to a certain extent; this hypothesis is reinforced by the dissimilarities noted above in the interaction with some animal cells. Comparative structural studies are needed to solve this point

The amino-acid composition (Table 5) of ERL shows several differences with those reported for other lectins of the genus *Erythrina* [5–13]. Performic acid oxidation revealed the presence of eight 1/2 Cys, which is unusual in these proteins; acidic and hydroxy aminoacids are found in large amounts and leu, phe, tyr, his and arg are present in lesser quantities than in other *Erythrina* lectins. The calculated M_r, based on the aminoacid composition, gives a value of 49 767 which correlates well with the M_r determined by gel filtration; discounting the 10% carbohydrate, the latter M_r is *ca* 55 800. The value $A^{1\%}_{1\,cm} = 16.043$ is slightly lower than that found for the *E. edulis* lectin [10] which is in accord with the smaller trp + tyr content of the *E. rubrinervia* lectin.

The lectin characterized in this study shows the gross

Table 5 Amino acid composition of the *E rubrinervia* lectin

	g AA/ 100 g protein	Calculated residues/mol	Nearest integer
Lys	1 09	22 0	22
His	0 29	5 6	6
Arg	0 40	6 6	7
Asp	2 92	65 3	65
Thr	1 11	28 3	28
Ser	2 40	71 0	71
Glu	3 19	63 7	64
Pro	0 87	23 1	23
Gly	1 04	46 9	47
Ala	0 59	21 4	22
Cys*	0 46	7 9	8
Val	0 75	19 6	20
Met†	0 38	6 0	6
Ile	0 67	16 6	17
Leu	0 76	17 4	18
Tyr	0 63	9 9	10
Phe	0 63	11 0	11
Trp‡		15 8	16

*Determined as $CySO_3$

†Determined as $MetSO_2$

‡Determined colorimetrically

Calculations are based on a M_r of 62 000 with 10% carbohydrate

structural features common to the lectins of the *Erythrina* genus [5–13] although a closer scrutiny of its behaviour and of the properties reported for several *Erythrina* lectins points to interesting differences among these proteins, i.e. variability of interaction with some animal cells (animal RBCs, lymphocytes), inability of some sugars to inhibit some lectins and variable subunit composition In view of the large number of available *Erythrina* lectins it is foreseeable that comparative studies will help to explain these differences and may contribute to our understanding of the reasons for the high degree of conservation of these proteins during evolution

EXPERIMENTAL

Materials Slightly outdated human blood was obtained from the Red Cross Blood Bank, Bogota Animal erythrocytes were supplied by the Hematology Laboratory of the Veterinary Faculty Proteins used as standards were all from Sigma Sugars were commercial products of the highest purity available All reagents were of analytical grade The seeds of *E rubrinervia* (voucher COL 126075) were collected at Caqueza, Cundinamarca

Isolation and purification The seeds were ground in a mill to 40–60 mesh The flour was deffatted (8 hr) with petrol and extracted with 1% NaCl (1 10, w/v) at room temp with stirring for 8 hr The extract was filtered through cheese-cloth and clarified by centrifugation (6000 rpm, 20 min, 4) The rest of the procedure was as described in ref [10] Parallel extractions were made with a buffer of the following composition 4 5 mM K-Pi, 240 mM NaCl, 5 mM PMSF, 2 5 mM EDTA, 2 5 mM EGTA, 1 mM ε-NH_2 caproic acid, 0 05 mM TLCK, 0 025 mM TPCK, pH adjusted to 7 2 The conditions for the extraction and purification were as described above

Protein M_r, electrophoresis, aminoacid and pI determinations were performed by the methods cited in ref [10]

Hemagglutination and inhibition assays were done as in ref [11]

Neutral sugar was estimated by the method of ref. [20] using D-glucose as standard Analyses were done both on native lectin and on lectin treated as follows The pure protein was dissolved (*ca* 2 8 mg/ml) in 50 mM NH_4OAc and 1 2 ml of this soln was applied on a column (87 × 2 cm) of Sephadex G-25 equilibrated with 4 M guanidine HCl The protein peak, localized by its A_{280}, was dialysed exhaustively vs 100 mM NH_4OAc Protein and carbohydrate content of the retentate were determined

Metal content The native protein was dissolved in 0 5% NaCl and dialysed exhaustively against the same solns An aliquot (*ca* 10 ml) of the dialysed protein soln was dialysed successively against 250 ml of the following solns 50 mM EDTA (× 3), 1 M HOAc (× 4) and 0 5% NaCl (× 4) The analyses were done on a Varian AA-475 atomic absorption spectrophotometer using as blank the respective last diffusates All solns were prepared in deionized H_2O

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