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A COMPARATIVE STUDY ON LECTINS FROM FOUR *ERYTHRINA* SPECIES

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Key Word Index—*Erythrina arborescens*; *E. indica*; *E. lithosperma*; *E. suberosa*; Leguminosae; lectins; characterization; comparison.

Abstract—A comparison of some physicochemical and structural properties of the lectins from the seeds of different species of the genus *Erythrina* is presented. The amino acid compositions of *E. indica*, *E. arborescens*, *E. lithosperma* and *E. suberosa* lectins are closely similar and resemble those of *E. cristagalli* [*Eur. J. Biochem.* (1982) **123**, 247–252] and *E. corallodendron* [*Can. J. Biochem.* (1981) **59**, 315–320]. They are rich in acidic and hydroxy amino acids and poor in sulphur containing amino acids. All contain valine as the only *N*-terminal amino acid. They are glycoproteins containing high mannose type complex oligosaccharide chains. In addition to mannose they contain arabinose, xylose, fucose, glucose and galactose. Glucosamine is the amino sugar present. *E. indica*, *E. arborescens* and *E. lithosperma* lectins bind to Concanavalin A-Sepharose but not to lentil and pea lectin-Sepharose indicating the presence of terminal non-reducing α-D-mannose and/or internal 2-*O*-α-linked mannose residues in these lectins, and the absence of α(1 → 6) linked L-fucose residues in the core regions of the oligosaccharide moieties. These lectins are metalloproteins containing about 2 g atoms of Mn^{2+} and 3 g atoms of Ca^{2+}. The M_rs of *E. arborescens*, *E. lithosperma* and *E. suberosa* lectins are 58 000, 57 000 and 59 000, respectively. Each lectin consists of two noncovalently bound subunits which are of identical or very similar M_rs. The UV spectra of *E. indica*, *E. arborescens* and *E. lithosperma* lectins have similar features and in the presence of inhibitory sugars the absorbance at 278–281 nm and 290 nm increases in all cases indicating involvement of tryptophan residue(s) in sugar binding. The properties of *Erythrina* lectins show striking similarities and indicate close phylogenic relationships among these lectins.

INTRODUCTION

Lectins are cell-agglutinating proteins of nonimmune origin that bind to specific carbohydrate determinants without chemically modifying them [1, 2]. They are found in plants as well as in animals and invertibrates. However, the majority of the best studied lectins are from plants, and they have been widely used to explore the membrane properties of both normal and transformed cells [2–4]. The physicochemical properties and carbohydrate specificity of many of them are well established [3, 4]. However, the results so far available on lectins from different species of the same genus are not sufficient to suggest any correlation between taxonomic relatedness of plants and the carbohydrate specificity or immunologic properties of lectins derived from them. For instance, lectins from two *Phaseolus* species (viz. *P. vulgaris* and *P. lunatus*) and five *Vicia* species (viz. *V. faba*, *V. cracca*, *V. sativa*, *V. ervilia* and *V. graminea*) differ widely in physicochemical as well as in cell-agglutinating and carbohydrate binding properties [cf. 4]. On the other hand, *Lathyrus sativus* [5] and *Lathyrus odoratus* [6] lectins appear to be very similar. These results emphasize the need for a closer and more extensive examination of the properties of lectins from different species of the same genus.

In a previous communication [7] we reported the purification of lectins from four *Erythrina* species, namely *E. indica*, *E. arborescens*, *E. lithosperma* and *E. suberosa*, and showed that these lectins were strikingly similar in their carbohydrate binding, cell agglutinating and immunochemical properties. The results obtained on lectins purified from *E. corallodendron* [8] and *E. cristagalli* [9] lend further support to our findings, which show that all *Erythrina* lectins so far purified are D-galactose/*N*-acetyl-D-galactosamine specific, and similar in respect of their hemagglutinating activities. Previously we presented some physicochemical properties of *E. indica* lectin [7]. In this paper we present and compare the amino acid and carbohydrate compositions of four *Erythrina* lectins. We found that these lectins are metalloproteins containing about 2 g atoms of Mn^{2+} and 3 g atoms of Ca^{2+} per mole of protein. The presence of Mn^{2+}, a paramagnetic ion, will enable us to use a variety of spectroscopic methods to study the molecular properties and carbohydrate binding activities of these lectins. The UV spectra of the lectins in the absence and presence of inhibitory sugars are also presented. Comparison of the properties of these lectins with those of *E. corallodendron* [8] and *E. cristagalli* [9] lectins show striking similarity among all *Erythrina* lectins.

RESULTS

Amino acid composition

Table 1 shows the amino acid compositions of *E. arborescens*, *E. lithosperma* and *E. suberosa* lectins

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Table 1. Amino acid composition of purified lectins

Amino acid	Residues per mol				
	*E. arborescens**	*E. lithosperma**	*E. suberosa**	*E. indica*†	*E. cristagalli*‡
Lys	19	22	22	18	20
His	10	10	11	10	8
Arg	10	11	19	11	11
Asp	57	58	51	63	62
Thr	39	39	38	44	43
Ser	49	41	43	51	47
Glu	57	55	42	61	55
Pro	31	32	32	34	39
Gly	37	35	67	38	39
Ala	30	34	47	40	40
Half-cys	0	0	0	0	0
Val§	41	37	48	42	42
Met	4	2	0	6	6
Leu	32	36	32	37	37
Ile§	23	26	21	29	30
Tyr	19	17	13	22	20
Phe	25	25	21	29	28
Try‖	10	10	n.d.	13	11
Total	493	490	507	548	538

*Based on the assumption that *E. arborescens* (M_r 58 000), *E. lithosperma* (M_r 57 000) and *E. suberosa* (M_r 59 000) lectins contain 10, 10 and 11 histidine residues per mol, respectively.

†From Bhattacharyya *et al.* [7].

‡From Iglesias *et al.* [9].

§Obtained in 72 hr hydrolysate.

‖Determined spectrophotometrically [29].

n.d., Not determined.

determined as average values from the results of duplicate analyses of each hydrolysate. The amino acid compositions of *E. indica* [7] and *E. cristagalli* [9] lectins are included in this table for comparison. The results show that all *Erythrina* lectins have very similar amino acid composition. They are rich in aspartic acid, glutamic acid, serine, threonine and valine. In *E. indica*, *E. arborescens*, *E. lithosperma* and *E. cristagalli* lectins, the acidic amino acid residues comprise 21.7–23.1 % of the total number of amino acid residues, the basic amino acid residues 7.1–7.9 %, the apolar amino acid residues 38.1–40.1 % and tyrosine and tryptophan together 5.5–6.4 %. Cysteine is absent from all of them, and the methionine content is very low. However, the amino acid composition of *E. suberosa* lectin appears to be slightly different. It is devoid of methionine and exhibits a lower content of acidic amino acids (18.3 %), and a higher content of basic (8.8 %) as well as apolar amino acids (46.6 %), particularly glycine and alanine, compared to the other four *Erythrina* lectins.

The *N*-terminal amino acid residue of *E. arborescens* and *E. lithosperma* lectins is valine, as was also found for *E. indica* [7] and *E. cristagalli* [9] lectins.

*Abbreviations: Con A, native Concanavalin A; PBS, 10 mM sodium phosphate buffer, pH 7.0, containing 0.15 M NaCl; EDTA, disodium ethylenediaminetetraacetate.

Carbohydrate composition

Table 2 shows that the carbohydrate compositions of the four *Erythrina* lectins are very similar. Glucosamine is the only amino sugar present in all of them. These lectins also contain arabinose, xylose, fucose glucose, galactose and mannose but no sialic acid residue. Mannose is the most abundant sugar and constitutes 39.5–48 % of the total number of sugar residues in these lectins. However, arabinose and glucose are absent in *E. lithosperma* and *E. cristagalli* lectins [9]. Similar monosaccharide residues have been shown to be present in a number of plant lectins [3, 4]. The carbohydrate composition of *E. suberosa* lectin could not be determined due to the lack of material.

Binding to Concanavalin A, lentil and pea lectins

Lectins from *E. indica*, *E. arborescens* and *E. lithosperma* give strong precipitin arcs with Concanavalin A in agar gel double diffusion experiments in PBS*. The arcs dissolved slowly in 0.1 M methyl α-D-mannopyranoside indicating the specificity of the reaction. Similar reactions with Con A were also observed with lima bean [10], soybean and wax bean [11] lectins. The specific binding of the three *Erythrina* lectins to Con A is further corroborated by the results (Fig. 1) of chromatography of these lectins on a Con A-Sepharose column. They bind to Con A-Sepharose column and are eluted slowly by 0.5 M D-glucose indicating strong binding [12]. None of these *Erythrina* lectins, however, give any precipitin arc with lentil or pea lectins nor bind to lentil or pea lectin-Sepharose columns.

Table 2. Carbohydrate composition of purified lectins

Sugar	Residues per mol			
	*E. indica**	*E. arborescens**	*E. lithosperma**	*E. cristagalli*†
Ara	3.3	3.7		
Xyl	7.4	3.7	2.8	2.0
Fuc	6.1	2.6	1.9	1.9
Glu	0.8	0.5		
Gal	1.7	2.1	2.1	
Man	17.6	9.0	8.3	7.1
Glu NH_2	5.8	1.2	1.8	3.8
Wt % of sugar	11.2	6.7	5.7	4.5

*Assuming the M_rs of *E. indica*, *E. arborescens* and *E. lithosperma* lectins are 68 000, 58 000 and 57 000 respectively.

†From Iglesias *et al.* [9].

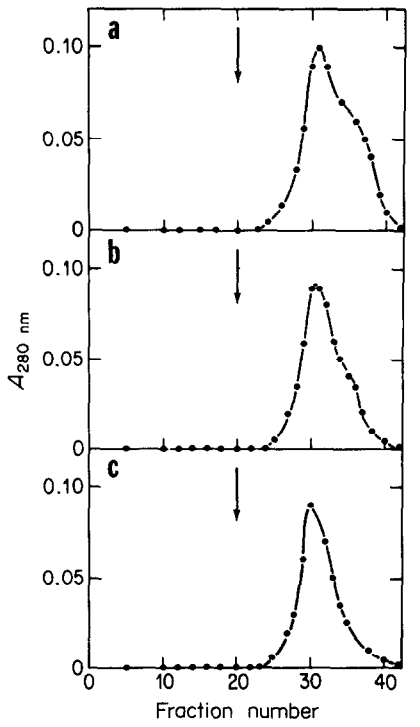

Fig. 1. Elution profile of (a) *E. indica*, (b) *E. arborescens* and (c) *E. lithosperma* lectins from Con A-Sepharose column. One ml fractions were collected at 10 ml/hr. The arrows show where elution was begun with 0.5 M D-glucose.

Molecular weight

The M_rs of *E. arborescens* and *E. lithosperma* lectins obtained by gel-filtration were 61 000 and 60 000, respectively. The values calculated from amino acid and carbohydrate compositions were 58 000 and 57 000, respectively. The values found by the two methods are in excellent agreement. From the amino acid composition and the total carbohydrate content of 6.8% [7], the M_r of *E. suberosa* lectin was found to be 59 000. The M_r of *E. indica* lectin determined by different methods was reported to be 65 000–68 000 [7].

Atomic absorption analysis

The results of metal determination by atomic absorption analysis indicate that *E. indica*, *E. arborescens* and *E. lithosperma* lectins are metalloproteins containing 1.8–2.3 g atoms of Mn^{2+} and 2.9–3.5 g atoms of Ca^{2+} per mol of lectin. None of the proteins contain Mg^{2+} or Zn^{2+}. 'Demetallization treatment' [10] of these lectins did not alter their metal content.

Hemagglutination assays

Minimum hemagglutinating doses [13] of *E. arborescens* and *E. lithosperma* lectins after 'demetallization treatment' against human blood group O erythrocytes were found to be the same as those of the respective native lectins, the values being 7.8 and 15.6 μg/ml, respectively. A similar result was reported previously for *E. indica* lectin [7].

UV spectra

Figure 2 shows the UV spectra of *E. indica*, *E. arborescens* and *E. lithosperma* lectins as such, and also in the presence of 0.2 M lactose, an inhibitory sugar. The spectra of these lectins are similar with maxima at 278–281 nm and 290 nm. The presence of lactose causes an increase in absorbance of each lectin, maximum perturbations occuring at the maxima. Similar results were obtained in the presence of 0.2 M D-galactose. The effects are specific since no change in spectra was observed in the presence of 0.2 M D-glucose which is a noninhibitory sugar. Similar results were also found with *E. cristagalli* lectin [9]. The results indicate that the environments of the aromatic amino acid residues are similar in these lectins, and that there is a change in the environment of tryptophan residue(s) upon sugar binding [14].

DISCUSSION

The similarity in physicochemical and biological properties of different *Erythrina* lectins appears to be very close. The studies reported previously [7–9] on lectins of six species of the genus *Erythrina*, e.g. *E. indica*, *E. arborescens*, *E. lithosperma*, *E. suberosa*, *E. cristagalli* and *E. corallodendron* show that all of them are specific

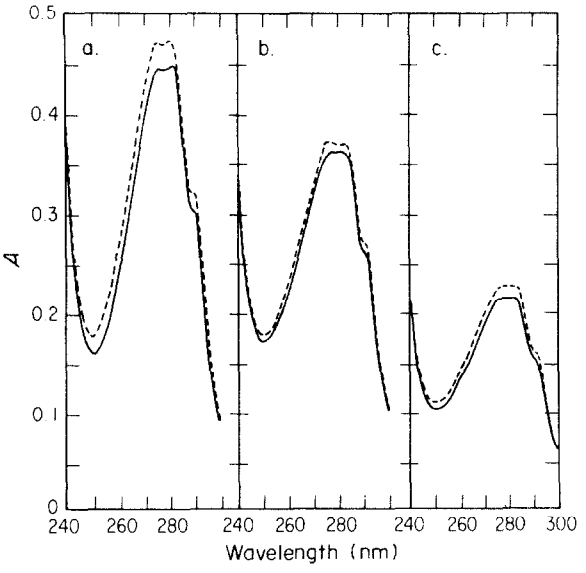

Fig. 2. UV spectra of *Erythrina* lectins in PBS in the absence (—) and presence of 0.2 M lactose (– –). The protein concentrations in the absence and presence of sugars were the same. (a) *E. indica* (0.40 mg/ml); (b) *E. arborescens* (0.35 mg/ml); (c) *E. lithosperma* (0.25 mg/ml).

towards D-galactose and its derivatives, *N*-acetyl-D-galactosamine being a better inhibitor than D-galactose, and with the exception of *E. indica* lectin they show slight preference towards α-anomers. Phenyl and *p*-nitrophenyl derivatives have been found [7, 9] to be better inhibitors than the corresponding methyl derivatives indicating the importance of the hydrophobic contribution of the aromatic aglycone in the sugar binding activity of *Erythrina* lectins. This point has also been established by Kaladas *et al.* [15] for *E. cristagalli* lectin. The hemagglutinating activity of *Erythrina* lectins have also been found to be similar [7–9] in being nonspecific towards human erythrocytes with some preference for blood group O. *Erythrina cristagalli* [9] and *E. corallodendron* [8] lectins are mitogenic towards human T lymphocytes. The corresponding data for the four other lectins are not available, but, in view of the striking similarity among *Erythrina* lectins reported here and elsewhere [7–9, 16], they will be mitogenic.

The results presented in Tables 1 and 2 clearly indicate the extreme similarity in amino acid and carbohydrate compositions of the *Erythrina* lectins studied here. While this manuscript was in preparation Lis *et al.* [16] reported a comparative study on nine other *Erythrina* lectins. The results of amino acid analysis of these authors are very similar to our results. Such similarity among all *Erythrina* lectins studied so far is striking and calls for closer attention to their phylogenic relationship.

Precipitation of *E. indica*, *E. arborescens* and *E. lithosperma* lectins with Con A and strong binding of these lectins to Con A-Sepharose column (Fig. 1) indicate the presence in their oligosaccharide moieties of branched structures [17] containing terminal non-reducing α-D-mannose and/or internal 2-*O*-α-linked D-mannose residues which are potential receptors for Con A [18]. The presence of internal 2-*O*-α-linked D-mannose residues were shown to be necessary for binding of glycopeptides to Con A-Sepharose [19, 20]. Kornfeld *et al.* [12] and Debray *et al.* [21] reported that an L-fucose residue attached to the asparagine-linked 2-actamido-2-deoxy-β-D-glucose residue through α(1 → 6) linkage was essential for high affinity binding of glycopeptides to lentil and pea lectin-Sepharose columns. The lack of binding of these lectins to lentil and pea lectins indicate the absence of such structure in their oligosaccharide chains. The other possibility that all but one α-mannose residues are substituted at C-2 and C-4 [12, 22] is ruled out because such a structure will not bind to Con A-Sepharose [20].

All *Erythrina* lectins are metalloproteins containing Mn^{2+} and Ca^{2+}. Similar results were reported for *E. indica* lectin by Horejsi *et al.* [23] who found 2.5 g atoms of Mn^{2+} and 1.3 g atoms of Ca^{2+} per mol of lectin. Though the Mn^{2+} content reported by these authors is nearly the same as what we obtained, the Ca^{2+} content found by them is less than half of our result. Dialysis against 0.1 M EDTA and 1 M acetic acid was found to remove metal ions from many lectins, including Con A, and lentil, pea, soybean, lima-bean and waxbean lectins with concomitant loss of activity [10, 24–26]. But similar treatment did not alter the metal content and hemagglutinating activity of *Erythrina* lectins studied here, indicating unusually strong metal binding to these lectins. Similar results were found by Lonnerdal *et al.* [27] for some other lectins.

The subunit M_rs of *E. lithosperma* lectin were reported to be 26 000 and 28 000, and those of *E. arborescens* and *E. suberosa* lectins to be 28 000 [7]. Comparison of the subunit M_rs with the M_rs of the respective intact proteins determined here shows that these lectins consist of two subunits. Since cysteine is absent in these lectins (Table 1), the subunits are noncovalently bound. Similar results were reported for *E. indica* [7] and *E. cristagalli* [9] lectins.

Perturbation of the UV spectra of *Erythrina* lectins in the presence of inhibitory sugars (Fig. 2) indicate involvement of tryptophan residues in sugar binding. This would explain the considerable enhancement of inhibition of hemagglutinating activity of *Erythrina* lectins [7, 9] caused by the hydrophobic contributions of aromatic aglycones of phenyl and *p*-nitrophenyl galactosides.

The close similarity among *Erythrina* lectins studied here as well as those reported in the literature [7–9, 16] in respect of their molecular properties and carbohydrate binding specificity shows a high degree of conservation of lectins during evolution and indicates an important, though still unknown, function of these proteins in plants. Further studies on the molecular properties and carbohydrate binding specificity of *E. indica* lectin are in progress.

EXPERIMENTAL

Materials. Dansyl chloride, dansyl amino acids and the silylating reagent, Tri-sil 'Z', were purchased from Pierce Chemical Company, U.S.A. Mannitol was a product of Sarabhai-Merck, India. Standard sugars and proteins were products of Sigma Chemical Co., U.S.A. Con A was purchased from Miles-Yeda Ltd., Israel. All other reagents were of analytical grade and were used as received.

Purification of lectins. The lectins from seeds of *E. indica*, *E. arborescens*, *E. lithosperma* and *E. suberosa* were extracted and purified according to the procedure described previously [7].

Lentil [28] and pea [29] lectins were purified according to the procedures described in the literature. The proteins were dialysed free of salt and stored lyophilized.

Amino acid composition. Amino acid analyses were performed in a Beckman Multichrom liquid column chromatograph (Beckman Instrument, Munich, West Germany) according to Spackman *et al.* [30]. Weighed samples of proteins corrected for moisture content were hydrolysed with 6 N HCl at 110° for 24, 48 and 72 hr in sealed evacuated tubes. Duplicate analyses were done with each hydrolysate. Values for serine and threonine were obtained by extrapolation to zero-time hydrolysis. Tryptophan was estimated spectrophotometrically [31]. *N*-Terminal amino acids were determined by the dansyl chloride method [32].

Carbohydrate composition. Neutral sugars were determined by GC in a Pye Unicam, Model GCD gas chromatograph with FID using a glass column (190 × 0.4 cm) packed with 5% SE-30. The column was temp. programmed from 140 to 200° at 1°/min and eluted by O_2 free N_2 (60 ml/min). Weighed samples of proteins and known monosaccharides were treated according to Chambers and Clamp [33] with 1 M anhydrous methanolic HCl for 24 hr at 100°. The methyl glycosides obtained were silylated with Tri-sil 'Z' reagent after re-*N*-acetylation with Ac_2O and drying over P_2O_5. Mannitol was used as the internal standard.

Amino sugars were determined according to Spiro [34] in the amino acid analyser with samples hydrolysed at 110° in sealed evacuated tubes with 4 N HCl for 6 hr.

Chromatography on Con A, lentil and pea lectin-Sepharose columns. Con A, lentil and pea lectin-Sepharose were prepared according to the standard procedure [35, 36]. About 0.7 mg of each *Erythrina* lectin was loaded onto the column (13 × 0.5 cm) equilibrated in PBS. After washing the column with about 20 ml PBS the adsorbed lectin was eluted with 0.5 M D-glucose in the same buffer.

Gel-filtration experiments. These were done [37] for the determination of the M_rs of *E. arborescens* and *E. lithosperma* lectins on a Sephadex G-200 column (80 × 1.8 cm) in 10 mM NaPi buffer, pH 7.0, containing 0.5 M NaCl using hexokinase (M_r 96 000), bovine serum albumin (M_r 68 000), ovalbumin (M_r 43 000), α-chymotrypsinogen A (M_r 25 000) and lysozyme (M_r 14 000) as standard proteins.

Atomic absorption analysis. Weighed samples of proteins were dissolved in 1 ml (about 5 mg/ml) of 0.1 M KOAc buffer, pH 6.4, containing 0.9 M KCl and 10 μM EDTA, and dialysed overnight against 1 l. of the same buffer to remove any nonspecifically bound metal ions [38]. The analyses were done on these samples with a Perkin–Elmer Model 603 atomic absorption spectrophotometer following Brewer *et al.* [39].

Demetallization of lectins. The lectins were subjected to 'demetallization treatment' at room temp. with 0.1 M EDTA and 1 M HOAc according to the procedure of Galbraith and Goldstein [10].

UV spectra. These were taken at room temp. in PBS in the absence and presence of simple sugars in the range 240–300 nm.

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