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CHARACTERIZATION OF A LECTIN FROM THE SEEDS OF *ERYTHRINA VESPERTILIO*

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Key Word Index—*Erythrina vespertilio*; Leguminosae; lectin; affinity purification; chemical characterization; sugar specificity.

Abstract—A lectin was isolated from the seeds of *Erythrina vespertilio* by affinity chromatography on lactose-Sepharose 6B. The lectin has an M_r of 59 000 and consists of two non-covalently associated subunits ($M_r \sim 30\,000$). The lectin is devoid of cysteine but has six methionine residues/mol and a neutral sugar content of 9.7%. The carbohydrate composition was mannose, *N*-acetylglucosamine, fucose, xylose and galactose in amounts of 15.0, 4.0, 1.0, 5.0 and 25 mol/59 000 g, respectively. Alkaline gel electrophoresis and isoelectric focusing showed that the affinity purified lectin consists of a family of isolectins. Valine was the only *N*-terminal amino acid found and the *N*-terminal sequence was homologous with that found for other legume lectins. The lectin was inhibited by galactosyl containing carbohydrates; *p*-nitrophenyl-β-galactoside was the best inhibitor and the lectin showed a slight preference for β-galactosides. Comparison of its properties with those of other *Erythrina* lectins shows that most of the lectins of this genus are closely related.

INTRODUCTION

Lectins are carbohydrate-binding, cell agglutinating proteins which occur in many bacteria, plant and animal tissues [1, 2]. The plant lectins display a wide variety of unique biological properties [3] and they often show a remarkable degree of specificity in the agglutination of erythrocytes from various sources.

Lectins are found in the seeds of many plants, especially in the Papilionoideae subfamily of the Leguminosae. Hemagglutinin activity has been reported in at least 14 species of the genus *Erythrina*, a legume of subfamily Papilionoideae [4–6]. A number of *Erythrina* lectins have been isolated and characterized [7–12] and recently Lis *et al.* [13] compared the properties of the lectins from nine *Erythrina* species of widely different origins.

Erythrina vespertilio L., the batswing coral pea, a native to Australia, grows in many arid environments with profuse yields of large seeds and has been proposed as a possible arid zone crop [14]. *Erythrina vespertilio* extracts contain a potent hemagglutinin activity; the present work describes the isolation and characterization of the lectin and compares its properties with other recently described *Erythrina* lectins [7–13].

RESULTS AND DISCUSSION

The hemagglutinin activity present in seed extracts of *E. vespertilio* was quantitatively adsorbed to lactose-Sepharose 6B and the lectin was eluted from the column as a sharp peak when 0.3 M galactose was added to the buffer. The yield of lectin was 2.2 mg per g of defatted seed meal. Similar yields are reported for other affinity purified *Erythrina* lectins [7, 11–13]. On gel filtration in PBS, *E. vespertilio* lectin yielded a symmetrical protein/agglutinin activity peak but the protein was retarded by the agarose matrix of the Ultrogel AcA 44 (apparent $M_r \sim 50\,000$) as has been reported for a number of galactose binding lectins [15].

Alkaline PAGE at pH 8.8 of the affinity purified lectin showed a broad band containing three discrete components. On isoelectric focusing 2–3 major protein bands and several minor bands (pI 4.8–5.3) were observed indicating that *E. vespertilio* lectin occurs as a family of isolectins as reported for lectins of other *Erythrina* species [10, 12, 14]. On SDS-PAGE, *E. vespertilio* lectin migrated as a single sharp band of apparent M_r 32 000. Most *Erythrina* lectins [10, 12, 13] have been reported to contain a single polypeptide subunit ($M_r \sim 29\,000$) with only several exceptions (*E. indica* [7], *E. lithosperma* [10] and *E. cristagalli* [11]) which yield two polypeptide subunits of slightly different size on SDS-gels (M_r 26 000–30 000).

Characterization of E. vespertilio *lectin*

Analytical ultracentrifugation showed that the lectin was homogeneous and an M_r of 59 000 ± 2000 was found. This result and the apparent subunit M_r of 32 000 show that the lectin is a dimeric protein composed of two non-covalently associated polypeptides. The apparent overestimation of the subunit M_r is consistent with the glycoprotein nature of the lectin. The molecular properties of *E. vespertilio* lectin closely resemble those of the *Erythrina* lectins described previously [7, 11–13].

The amino acid composition of *E. vespertilio* lectin (Table 1) is characterized by a high content of acidic and hydroxy amino acids, six methionine residues and an absence of cysteine similar to that reported for other *Erythrina* lectins [7, 11, 13]. However, the composition of *E. edulis* lectin [12] differs markedly from that of the other *Erythrina* lectins, in particular the values reported for lysine and phenylalanine and it may be advisable to

Table 1. Amino acid composition of the *E. vespertilio* lectin

Amino acid	*E. vespertilio**	*E. cristagalli*†	*E. indica*‡	*E. edulis*§
Lys	22.3 (22)	20	18	89
His	11.9 (12)	8	10	14
Arg	12.5 (13)	11	11	7
Asp	65.1 (65)	62	63	52
Thr	42.6 (43)	43	44	33
Ser	47.8 (48)	47	51	35
Glu	52.3 (52)	55	61	49
Pro	38.5 (39)	39	34	36
Gly	40.0 (40)	39	38	32
Ala	42.8 (43)	40	40	33
Cys	0 (0)	0	0	0
Val	43.4 (44)	42	42	33
Met	5.2 (6)	6	6	3
Ile	30.9 (31)	30	29	20
Leu	37.7 (38)	37	37	26
Tyr	19.5 (20)	20	22	18
Phe	28.4 (28)	28	29	1
Trp	8.5 (9)	11	13	17

*Results are expressed as residue/mol and are based on an M_r of 59000 with 9.7% carbohydrate. Figures in parentheses are nearest integers. Values for serine and threonine are extrapolated to zero-time hydrolysis.

†Taken from ref. [11].

‡From ref. [7].

§From ref. [12].

check this unusual composition. Like other *Erythrina* lectins, *E. vespertilio* lectin is a glycoprotein. Analysis for amino sugars yielded about 4.0 residues of glucosamine per molecule. Carbohydrate analysis showed that *E. vespertilio* lectin contained a total carbohydrate content of 9.7% with a composition of mannose, *N*-acetylglucosamine, fucose and xylose present in amounts of 15.0, 4.0, 1.0 and 5.0 mol per mol of lectin, respectively. The lectin also contained 25 mol of galactose per mol, a sugar which is not found in any other *Erythrina* lectin so far described. Galactose was not found in affinity purified *P. tetragonolobus* lectins [16] analysed as controls.

Sequence analysis of *E. vespertilio* lectin in the sequenator yielded a single amino terminal valine and provided sequence data for 28 cycles. There was no evidence of heterogeneity in the amino terminal region of the molecule to account for the charge heterogeneity observed on isoelectric focusing suggesting the possibility that this heterogeneity may be due to either the carbohydrate moiety or charge differences within the polypeptide chain. *Erythrina vespertilio* lectin shows extensive homology with the *N*-terminal sequences reported for other *Erythrina* lectins [13] and other legume lectins as illustrated in Fig. 1 in agreement with the proposal that all Leguminosae lectins have evolved from a common ancestral precursor [17].

Erythrina vespertilio lectin showed no erythrocyte specificity and agglutinated native and trypsinized human (type A, B and O) and rabbit erythrocytes. The trypsin treatment of the erythrocytes increased the hemagglutinin titre by a factor of four. The minimum concentration of lectin to give a positive hemagglutinination reaction was ~ 3.0 μg/ml.

The agglutination activity of *E. vespertilio* lectin was effectively inhibited by all carbohydrates containing galactosyl residues. The inhibition of *E. vespertilio* lectin agglutination by a number of sugars (Table 2) is compared with results reported for *E. cristagalli* [11], *E. edulis* [12] and soybean [18] lectins. While methyl-α-D-galactoside was a better inhibitor than methyl-β-D-galactoside, a

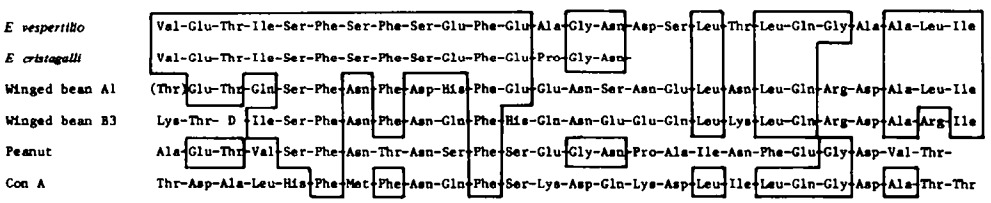

Fig. 1. Amino-terminal sequence of *E. vespertilio* lectin compared with the sequences of *E. cristagalli* lectin [13], winged bean acidic lectin A1 [28], winged bean basic lectin B3 [18], peanut lectin [29] and concanavalin A (residues 123–148) [29]. Residues identical with those in *E. vespertilio* lectin are boxed to illustrate the extent of homology. D denotes deletion introduced to maximize homology.

Table 2. Inhibition of hemagglutination activity of *E. vespertilio* lectin by various sugars

	Minimum inhibitory concentration (mM)*			
	E. vespertilio lectin*	*E. cristagalli* lectin†	*E. edulis* lectin‡	Soybean lectin§
N-Acetyl-D-galactosamine	0.78	6.5	5.3	0.09
D-Galactose	3.90	13.5	26.9	3.13
D-Galactosamine	15.60	20.0	105.0	6.25
D-Fucose	15.60	18.0		12.50
Methyl-α-D-galactoside	1.95	4.8	6.1	1.56
Methyl-β-D-galactoside	3.90	13.5	12.4	1.56
p-Nitrophenyl-α-D-galactoside	0.39	4.0		0.39
p-Nitrophenyl-β-D-galactoside	0.19	2.0	3.2	0.78
Thiodigalactoside	0.98			
Melibiose	1.95		14.7	1.56
Raffinose	3.90	7.2	54.5	3.13
Stachyose	7.80			
Lactose	0.49	2.0	1.8	3.13
Lactobionic acid	1.95			
Lactulose	0.98			
3-*O*-β-Galactopyranosyl-D-arabinose	3.90			3.91

*Trypsinized human type O erythrocytes were used. The same values were obtained with trypsinized human type A and B erythrocytes and trypsinized rabbit erythrocytes. The concentration of lectin was such that two two-fold dilutions would give the end point agglutination in the absence of inhibitors. The following sugars were not inhibitory at 0.25 M; glucose, methyl-α-D-glucoside, *N*-acetylglucosamine, methyl-α-D-mannoside, *N*-acetyl-D-mannoside and L-fucose.

†From ref. [11].

‡From ref. [12].

§From ref. [18].

marginal preference for the β-anomer over the α-anomer was found with glycosides of D-galactosides which had aromatic and carbohydrate aglycons. Thus *p*-nitrophenyl-β-D-galactoside was the most effective inhibitor and the β-galactoside, lactose, was a better inhibitor than the α-galactoside, melibiose. This inhibition data is in general agreement with that reported for other *Erythrina* lectins [7, 11–13] suggesting that they share structurally related sugar binding sites. However comparison with soybean lectin (Table 2) illustrates the different specificities between the *Erythrina* lectin and other legume lectins inhibited by galactosyl residues.

This study has shown that the molecular properties and sugar specificity of the lectin from *E. vespertilio*, an Australian native, are very similar to that of the lectins of other *Erythrina* species [7, 10–13] obtained from widely separate geographic regions. These findings indicate that the structure of the *Erythrina* seed lectins has been strongly conserved during evolution. Similar conservation of structure has been also observed for the Kunitz type trypsin inhibitors isolated from *Erythrina* [19] and other legume seeds.

EXPERIMENTAL

The seeds of *E. vespertilio* were purchased from the Nindethana Seed Service, Narrikup, W.A. 6326. Divinyl sulphone was purchased from Fluka and Sepharose 6B was from Pharmacia. Sugars were purchased from Sigma Co. and British Drug Houses. Lactose-Sepharose 6B was prepared according to Allen and Johnson [20].

Isolation of lectin. Finely ground seed meal (5 g), defatted with petrol, was extracted for 4 hr at room temp. with 200 ml 0.05 M Tris–HCl–0.1 M NaCl, pH 8, containing 5% (w/v) polyvinylpyrrolidone. The extract was clarified by centrifugation and the supernatant was concd (Amicon Diaflo, YM-10 membrane) to 40 ml. A 10 ml aliquot of the concd extract was applied to a lactose-Sepharose 6B column (6.0 × 1.5 cm) equilibrated with 0.01 M phosphate–0.15 M NaCl, pH 7.1 (PBS). The adsorbed lectin was eluted with 0.3 M galactose-PBS. The lectin was dialysed exhaustively against deionized water and recovered by lyophilization. Gel filtration of the lectin was performed on a calibrated Ultrogel AcA 44 column (150 × 1 cm) equilibrated with PBS at a flow rate 8.4 ml/hr.

Erythrocyte agglutination and inhibition assays. The erythro-agglutination activity was determined by measuring the ability of the lectin to agglutinate trypsinized human (type A, B and O) and trypsinized rabbit erythrocytes as described [18]. The sugar inhibition studies were performed as described [18].

Electrophoresis. Gel electrophoresis was carried out in 3 mm slabs (7.5% (w/v) polyacrylamide) at pH 8.8 [21]. SDS-PAGE was carried out in 3 mm slabs (12% w/v polyacrylamide) by the method of Laemmli [22]. Gel isoelectric focusing was carried out in the pH range 3.5–9.5 using Ampholine (LKB) polyacrylamide gel plates according to the manufacturer's instructions.

M_r *determination.* The M_r of the lectin was determined in PBS by the meniscus depletion method of Yphantis [23] in a Beckman Model E ultracentrifuge equipped with Rayleigh interference optics. The partial sp. vol. of 0.73 ml g^{-1} was calculated from the amino acid composition [24]. The subunit M_r was estimated by SDS-PAGE.

Amino acid analysis. Analyses were performed with a modified Beckman Spinco 120C analyser. Protein samples (0.2–0.4 mg) were hydrolysed *in vacuo* in 5.7 M HCl at 110° for 24 and 72 hr. Tryptophan was determined after hydrolysis of the samples in 4 M methane sulphonic acid containing 0.2% (w/v) tryptamine at 115° for 24 hr [25].

Carbohydrate analysis. The neutral sugars were determined by GC after formation of alditol acetate derivatives [26] in the presence of myo-inositol as an internal standard. GC was carried out using a column of 3% SP 2340 on Supelcoport (86–100 mesh) with a temp. prog. from 200° to 235°. The amino sugar, glucosamine, was determined on the amino acid analyser after hydrolysis in 3 M methane sulphonic acid at 100° for 24 hr [27] with fluorophenylalanine as an internal standard.

Amino-terminal sequence analysis. The amino terminal sequence was determined on a sequenator using 0.1 μmol of protein and phenylthiohydantoin (PTH) derivatives were identified by high performance liquid chromatography.

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