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Isolation and characterization of isolectins from *Erythrina variegata* seeds

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ABSTRACT

Three isolectins were isolated from seeds of *Erythrina variegata* (Linn.) var. *Orientalis* by ion-exchange chromatography, followed by affinity chromatography on lactose–Sepharose 4B and acid-treated Sepharose 4B columns. The purified isolectins (EVLI, EVLII and EVLIII) are all specific for galactopyranosides and N-acetylgalactosamine, and their affinities for simple sugars are EVLIII > EVLII > EVLI. EVLI and EVLIII are homodimers made up of an A-subunit of molecular mass 36 000 and a B-subunit of molecular mass 33 000, whereas EVLII is a heterodimer composed of the A- and B-subunits. Upon treatment with trifluoromethansulphonic acid, the molecular masses of both subunits decreased to 31 000. Rechromatography of EVLII on the acid-treated Sepharose 4B column again produced the homodimeric lectins (EVLI and EVLIII). It is suggested that the constituent subunits of *Erythrina variegata* isolectins are eschangeable with each other *in vitro*.

INTRODUCTION

Lectins, which are carbohydrate-binding proteins or glycoproteins capable of agglutinating cells, are widely distributed in plants, particularly legumes [1], and also in vertebrates, invertebrates and microorganisms [2]. Some lectins have a cytotoxic or mitogenic effect on lymphocytes in addition to their cell-agglutinating effect. Such cellular events induced by lectins are initiated by the specific binding of lectins to the carbohydrate moiety on the cell membrane. In this context, attention has been paid to the specific interactions of lectin with carbohydrate as one of the biospecific systems to understand the rules of the molecular recognition of proteins. Because of their strict specificities for carbohydrate structure, lectins have also been used for isolation of glycoproteins, for analysis of complex carbohydrates and for studies of cell-surface architecture.

Recently, over a dozen lectins have been isolated from various species of the genus *Erythrina*, which are tropical or subtropical plants, and their structures and carbohydrate specificities have been studied comparatively [ref. 3 and references cited therein]. However, the data available for elucidation of the molecular mechanism of the binding of lectins to carbohydrates and the specific roles of lectins in plants are incomplete. To attain better understanding of the physiological roles and structure–function relationships of plant lectins, we have isolated three isolectins from seeds of *Erythrina variegata* (Linn.) var. *Orientalis* by affinity chromatography on an acid-treated Sepharose column and characterized their molecular properties. This is the first report describing the isolation and characterization of isolectins from the genus *Erythrina*.

MATERIALS AND METHODS

The seeds of *E. variegata* obtained in Okinawa were a generous gift from Dr. Nobuhiro Fukuda (University of Miyazaki, Miyazaki, Japan). DEAE-cellulose (DE-52) and Sepharose 4B were purchased from Whatman BioSystems (UK) and Pharmacia (Sweden), respectively. Lactose–Sepharose 4B and acid-treated Sepharose 4B were prepared according to the methods of Iglesias *et al.* [4] and Ersson *et al.*

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[5], respectively. Other chemicals used were of analytical grade.

Extraction of lectins from the defatted *E. variegata* meal, subsequent DEAE-cellulose column chromatography and lactose–Seharose 4B affinity chromatography were performed essentially as described by Fukuda *et al.* [3]. Affinity chromatography of the fractions eluted with 0.1 *M* lactose from the lactose–Sepharose 4B column was done on the acid-treated Sepharose 4B column (44 × 1.6 cm) equilibrated with 10 m*M* phosphate-buffered saline, pH 7.2 (PBS). The protein adsorbed to the acid-treated Sepharose 4B was eluted with PBS containing 0.1 *M* lactose.

The haemagglutinating activity of the lectin was assayed using human red blood cells, as described previously [3].

Deglycosylation with trifluoromethanesulphonic acid was performed exactly according to the method of Edge *et al.* [6].

Estimation of molecular mass was done by sodiumdodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli [7] using 15% acrylamide gel concentration and by gel filtration on a TSK G2000SW column equilibrated with PBS.

The amino acid composition of the protein was analysed on an amino acid analyser (Hitachi, 655-A type) after hydrolysis with 6 *M* hydrochloric acid containing 0.05% 2-mercaptoethanol in an evacuated sealed tube at 110°C for 24 h. The N-terminal amino acid sequence was determined by a PSQ-1 Shimadzu gas-phase protein sequencer.

RESULTS AND DISCUSSION

Isolation of isolectins

It has been well established, as described above, that a galactose-specific lectin is present in the seeds of the genus *Erythrina*. Likewise, purification of an extract of *E. variegata* seeds on a lactose–Sepharose 4B column provided the fraction with haemagglutinating activity (Fig. 1). However, SDS-PAGE analysis of the fractions bound to the lactose–Sepharose 4B gave two protein bands with estimated molecular masses of 36 000 and 33 000, which is inconsistent with the result reported previously [3]. Hence, the fraction was dialysed against PBS and then further purified on an acid-treated Sepharose 4B column equilibrated with PBS (Fig. 2). Chromatography on an acid-treated Sepharose 4B column separated the fraction into three proteins containing haemagglutinating activities. Thus, three isolectins were designated EVLI, EVLII and EVLIII in order of the elution from the column. SDS-PAGE analysis of the isolectins thus obtained gave single bands for EVLI and EVLIII with molecular masses of 36 000 and 33 000, respectively, while that of EVLII showed two bands at 36 000 and 33 000. On the other hand, estimation of the molecular mass of isolectins on a calibrated column TSK G2000SW indicated the molecular masses to be 52 500, 48 500 and 51 000 for EVLI and EVLII and EVLIII, respectively (Fig. 3). It was concluded by a combination of the results obtained from SDS-PAGE and gel filtration that isolectins EVLI and EVLII are

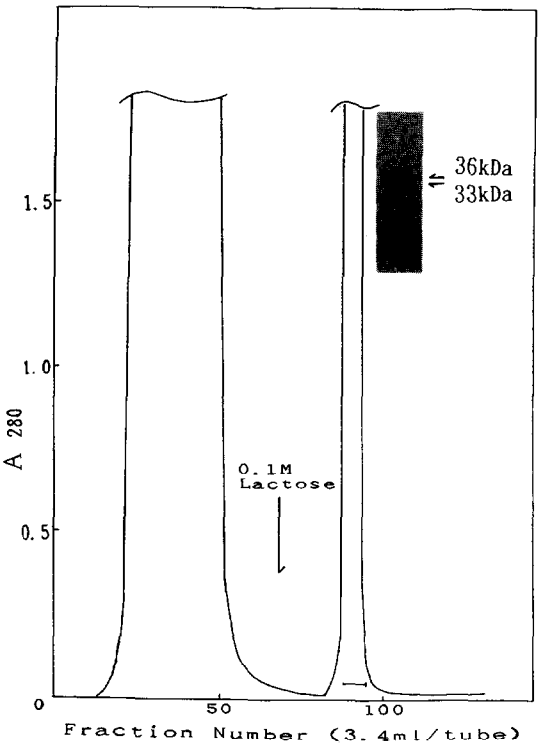

Fig. 1. Affinity chromatography of the fraction eluted with 0.15 *M* sodium chloride from a DEAE-cellulose column on a lactose–Sepharose 4B column (33 × 1.9 cm) and SDS-PAGE of the proteins adsorbed on the column. Elution was performed with PBS, followed by the same buffer containing 0.1 *M* lactose. The solid line indicates the fraction that was further purified on an acid-treated Sepharose 4B column. kDa = kilodalton.

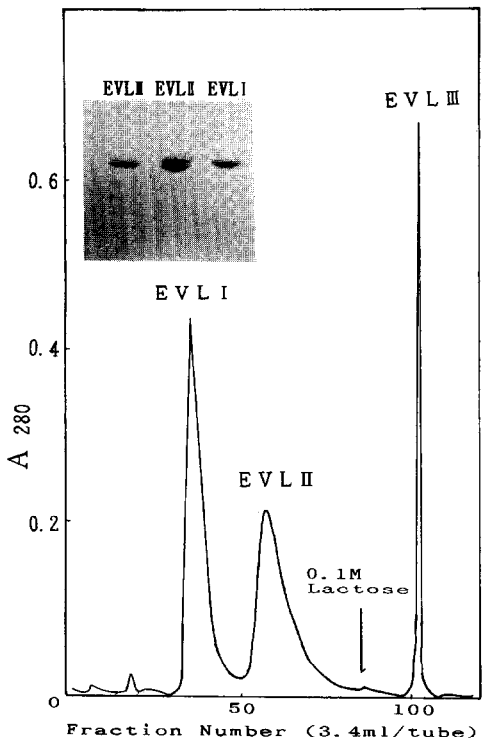

Fig. 2. Affinity chromatography of the fraction adsorbed by lactose–Sepharose 4B on an acid-treated Sepharose 4B column (44 × 1.6 cm) and SDS-PAGE of individual fractions. Elution was carried out by the same way as in the legend to Fig. 1.

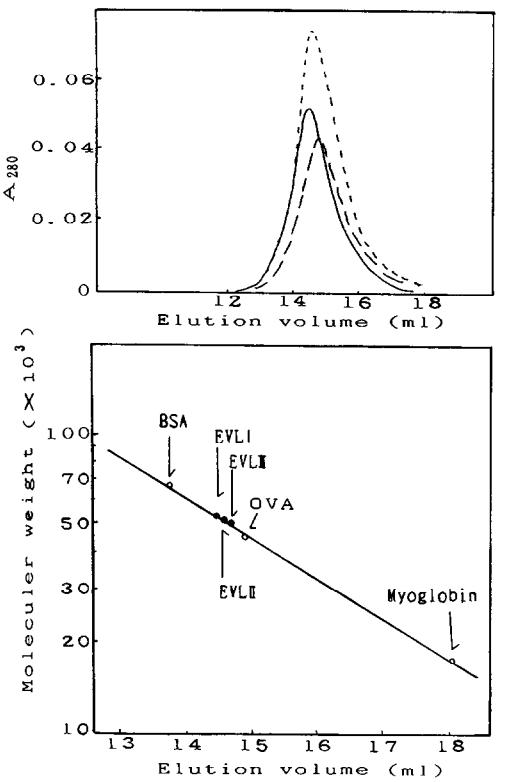

Fig. 3. Gel filtration of *E. variegata* isolectins, EVLI (—), EVLI (----) and EVLIII (- - -), on TSK G2000SW (600 × 7.5 mm). The column was developed with 50 m*M* sodium phosphate buffer, pH 7.0, containing 0.2 *M* sodium chloride at a flow-rate of 0.5 ml/min.

homodimers made up of a subunit of molecular mass 36 000 (A-subunit) and a subunit of molecular mass 33 000 (B-subunit), while EVLII is a heterodimer composed of an A-subunit and a B-subunit.

This finding raised the question as to whether the A- and B-subunits equilibrate with each other to form AA and BB, or whether isolectins composed of AA, AB and BB exist *de novo* in the mature seeds. To address this question, EVLII was rechromatographed on the acid-treated Sepharose 4B column under the same conditions. The result showed that the heterodimeric lectin EVLII again produced the homodimeric isolectins EVLI and EVLIII (data not shown). It was therefore demonstrated that the A- and B-subunits are easily exchangeable with each other *in vitro*.

Inhibition of haemagglutinating activity by carbohydrates

Inhibition of the haemagglutinating activities of isolectins by various sugars was tested (Table I). The overall specificities of carbohydrates for isolectins were found to be very similar, *i.e.*, as reported previously, all lectins were specifically inhibited by galactose and its derivatives. In particular *p*-nitrophenyl-β-N-acetyl-2-amino-2-deoxy-D-galactopyranoside and *p*-nitrophenyl-β-D-galactopyranoside were the most potent inhibitors of isolectins among the sugars tested. With respect of the affinities of isolectins for the carbohydrates, the affinities of EVLIII were, in general, 5–20 times higher than those of EVLI, and EVLII had moderate affinity compared with EVLI and EVLIII, a finding which is consistent with their affinities for acid-treated Sepharose 4B.

Biochemical properties of isolectins

To attain structural information about the A-

TABLE I

INHIBITION BY VARIOUS SUGARS OF THE HAEMAGGLUTINATING ACTIVITIES OF ISOLECTINS FROM *E. VARIEGATA* SEEDS

Sugar	Minimum concentration (m*M*) of sugar		
	EVLI	EVLII	EVLIII
D-Galactose	25	6.25	1.56
D-Fucose	57.1	28.57	10
2-Deoxy-D-galactose	66.7	25	10
N-Acetyl-D-galactosamine	6.25	3.57	1.04
Methyl-α-D-galactopyranoside	12.5	3.57	1.04
Methyl-β-D-galactopyranoside	16.7	6.25	2.5
Phenyl-β-D-galactopyranoside	5	1.79	0.521
p-Nitrophenyl-α-D-galactopyranoside	12.5	3.57	0.893
p-Nitrophenyl-β-D-galactopyranoside	0.893	0.893	0.446
p-Nitrophenyl-β-N-acetyl-2-amino-2-deoxy-D-galactopyranoside	1.79	0.391	0.156
Lactose	6.25	1.79	0.396
Melibiose	>25	>25	1.25
Lactulose	7.14	2.5	0.195
Raffinose	28.57	12.5	3.12

and B-subunits, isolectins EVLI and EVLIII were subjected to sequence analysis. Analysis of EVLI and EVLIII with the aid of a PSQ-1 Shimadzu gas-phase sequencer allowed us determine the sequence of the first N-terminal 40 and 29 residues, respectively, with the exception of position 17 in both proteins (Table II). The amino acid residues at position 17 in both proteins were tentatively assumed to be glycosylated asparagines because of the sequences around position 17 (Xaa-Leu-Thr) and the lack of phenylhydantoin (PTH) amino acids at the seventeenth step of Edman degradation. The sequence result indicated that A- and B-subunits share a highly similar protein structure. This finding led us to a postulate that the difference in the molecular masses between subunits A and B might be due to oligosaccharide chains.

To confirm this, EVLI, EVLII and EVLIII were deglycosylated with trifluoromethanesulphonic acid and then analysed by SDS-PAGE. As shown in Fig. 4, chemical deglycosylation of the three isolectins resulted in the generation of proteins of molecular mass 31 000. This result indicated that the difference in the molecular masses observed between the A- and B-subunits is mainly due to the presence in the A subunit of a carbohydrate moiety of molecular mass higher by 3000.

Table III shows the amino acid composition of subunits A and B. This was calculated on the basis of the molecular masses of the subunits (31 000).

TABLE II

THE N-TERMINAL SEQUENCES OF A- AND B-SUBUNITS OF ISOLECTINS FROM *E. VARIEGATA* SEEDS

	A-subunit		B-subunit	
Step	Amino acid	Yield (pmol)	Amino acid	Yield (pmol)
1	Val	842	Val	2951
2	Glu	643	Glu	2104
3	Thr	232	Thr	1906
4	Ile	746	Ile	2087
5	Ser	124	Ser	406
6	Phe	672	Phe	1872
7	Ser	105	Ser	437
8	Phe	584	Phe	1831
9	Ser	89	Ser	359
10	Glu	324	Glu	1093
11	Phe	480	Phe	1386
12	Glu	333	Glu	1181
13	Ala	459	Ala	1337
14	Gly	335	Gly	887
15	Asn	330	Asn	855
16	Asp	235	Asp	562
17	Xaa	–	Xaa	–
18	Leu	345	Leu	834
19	Thr	103	Thr	352
20	Leu	307	Leu	992
21	Gln	279	Gln	704
22	Gly	246	Gly	641
23	Ala	310	Ala	861
24	Ala	359	Ala	1108
25	Leu	272	Leu	746
26	Ile	261	Ile	659
27	Thr	80	Xaa	–
28	Gln	223	Gln	613
29	Ser	39	Ser	125
30	Gly	190		
31	Val	239		
32	Leu	123		
33	Gln	177		
34	Leu	119		
35	Thr	55		
36	Lys	126		
37	Ile	159		
38	Asn	139		
39	Gln	149		
40	Asn	140		

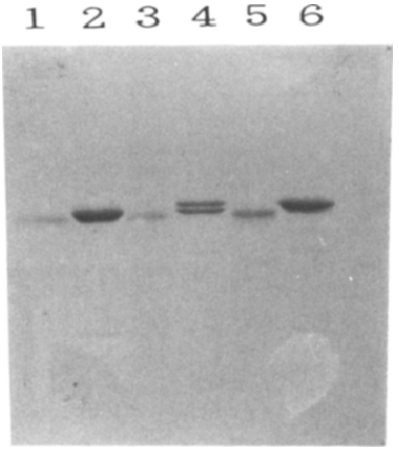

Fig. 4. SDS-PAGE of *E. variegata* isolectins before and after chemical deglycosylation with trifluoromethanesulphonic acid. Lanes: 1 = deglycosylated EVLIII; 2 = native EVLIII; 3 = deglycosylated ECLII; 4 = native EVLII; 5 = deglycylated EVLI; 6 = native EVLI.

The A- and B-subunits are composed of 255 and 253 amino acid residues, respectively, and again seem to be highly similar each other, having only a few amino acid differences.

The results presented in this paper indicate that isolectins isolated from *E. variegata* exhibit different affinities for acid-treated Sepharose 4B as well as carbohydrates, although they share a highly similar structure. Therefore, a more detailed comparative study on the protein structures of isolectins would give a clue to understanding the mechanisms of binding of lectins to carbohydrate. This study is now in progress in this laboratory.

TABLE III

AMINO ACID COMPOSITIONS OF A- AND B- SUBUNITS OF ISOLECTINS FROM *E. VARIEGATA* SEEDS

Amino acid	A-subunit	B-subunit
Asp	29.04 (29)	30.73 (31)
Thr	19.08 (19)	20.06 (20)
Ser	30.08 (30)	27.81 (28)
Glu	26.35 (26)	26.92 (27)
Pro	15.31 (15)	15.54 (15)
Gly	26.63 (27)	24.11 (24)
Ala	22.00 (22)	21.16 (21)
Val	16.13 (16)	16.54 (16)
Met	1.72 (2)	0.96 (1)
Ile	11.61 (12)	12.22 (12)
Leu	15.35 (15)	15.93 (16)
Tyr	8.78 (9)	8.40 (8)
Phe	12.21 (12)	12.23 (12)
Lys	9.17 (9)	9.55 (9)
His	6.23 (6)	5.66 (6)
Arg	5.47 (6)	5.66 (6)
Total	255	253

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