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A Basic Lectin from Bulbs of *Arisaema ringens*

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Summary

A lectin was purified from bulbs of *Arisaema ringens* Schoot, using DEAE-Cellulofine column chromatography and chromatofocusing. The purified lectin had the specific activity of 86,000 titer/mg protein/ml and its yield was 1.8%. The homogeneity of the lectin was confirmed by SDS-PAGE. Its isoelectric point was 8.2 and the molecular mass was 12.4kDa. It was only active toward trypsinized rabbit erythrocytes. It was deduced from the hemagglutination inhibition that the lectin recognized mannooligosaccharides and terminal N-acetyllactosamine.

Key words: *Arisaema ringens* Schoot, bulb, lectin, monocot.

Introduction

Many plant lectins are now available as biomedical and biological tools. Previously, 7 lectin families have been reported as plant lectins [1]. Of the 7 lectin families, mannose-binding lectins have been found in the monocot. However, *Araceae* lectins are different from other mannose-recognizing lectins in sugar-binding specificity. Allen first reported that Arum lectin recognized N-acetyllactosamine [2]. *Xanthosoma* lectin has been found to possess two sugar-binding sites [3]. One is mannose-binding site and the other one is N-acetyllactosamine-recognizing site. Furthermore, in *Araceae* lectins, many biological activities have been reported [4-14]. Therefore, *Arisaema* lectins are interesting ones. *Arisaema ringens* Schoot (Musashiabumi) is a plant living in Japan. Our preliminary study showed that the bulb of this plant had a strong hemagglutination activity.

In this study, we purified and characterized a lectin from bulbs of *Arisaema ringens* Schoot.

Materials and Methods

Materials

Bulbs of *Arisaema ringens* Schoot were collected in Mt.Yaeyama, Kagoshima, Japan. DEAE-Cellulofine A-200 and Polybuffer Exchanger PBE94 were purchased from Seikagaku Co. (Tokyo).

Saccharides

Sacharides, glycosides, and glycoproteins were obtained from Sigma Chemical Co. Quail ovomucoid was one of our laboratory collections. Asialoglycoproteins were prepared by

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desialylation with 0.1 M H_2SO_4 at 80℃.

Hemagglutination assay

Hemagglutination measurement was conducted in microtiter plates, in a final volume of 70 μl phosphate-buffered saline, pH 7.2 (PBS). Each well contained 50 μl of lectin solution and 20 μl of 4% (v/v) suspension of rabbit erythrocytes.

Agglutination was assessed after incubation for 1 h at room temperature, and hemagglutinating activity was expressed as titer, namely, the reciprocal of the highest dilution that gave a positive result. The specific hemagglutinating activity was defined as titer (mg lectin/ml)$^{-1}$.

Quantitation of protein and carbohydrate

Protein was quantified by the method of Lowry et al. [15] with bovine serum albumin as standard, and carbohydrate was quantified by the phenol-sulfuric acid method of Dubois et al. [16] with D-mannose as standard.

Electrophoresis

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a discontinuous system, as described by Laemmli [17]. Protein on all gels were stained with coomassie brilliant blue R-250.

Estimations of molecular mass

The molecular mass of native lectin was estimated by HPLC that was performed with a column of TSK-gel SWXL (7.8 x 300 mm). The solvent used was 0.1M sodium phosphate buffer, pH 7.0, at a flow rate of 0.5 ml min^{-1}.

Determination of N-terminal amino acid sequence

N-terminal sequencing was performed with a Procise 49XHT protein sequencer (Applied Biosystems-Perkin Elmer).

Purification procedure

Hemagglutinating activity was measured throughout all the purification procedures with rabbit erythrocytes. A total of 109 g of bulbs was homogenized and extracted with 1 l of PBS at 4℃ for 1h, and the homogenate was filtered through two layers of gauze and centrifuged at 12,000 × g for 15min. The solution was centrifuged to remove insoluble material. The supernatant solution was saturated with 100% ammonium sulfate. Protein precipitated was collected by centrifugation at 8,000 × g and dialyzed against 20 mM Na phosphate buffer, pH7.0.

Dialyzate was applied on a column of DEAE-Cellulofine (2.2 x 30 cm) previously equilibrated with the same buffer. Protein in the pass-through fractions was precipitated by 90% saturation of ammonium sulfate. Protein precipitated was collected by centrifugation and dissolved in 10 mM Na phosphate buffer, pH 8.0. The second DEAE-chromatography was conducted at pH 8.0. Hemagglutination activity was eluted by a linear gradient of 0-2.5M NaCl. Four fractions with hemagglutination activity were separately concentrated with ammonium sulfate.

Chromatofocusing was carried out for a fraction with the highest specific activity. The sample precipitated with ammonium sulfate was collected by centrifugation, and dialyzed against 25mM ethanolamine buffer. The dialyzate was applied onto a column of Polybuffer Exchanger PBE 94

(30ml), previously equilibrated with the same buffer, and the lectin was eluted with polybuffer 96, pH6.0. Finally, the column was washed with 2 M NaCl.

Results and Discussion

Purification of lectin from bulbs of *Arisaema ringens* Schoot - Figure 1 shows the final purification of the main lectin fraction by chromatofocusing. The isoelectric point of the main peak was 8.2. Table 1 summarizes the purification of the lectin. The specific activity of the lectin was 86,000 titer/mg protein/ml, and the yield was 0.9%. The final preparation was homogeneous on SDS-PAGE (Fig. 2). The molecular mass by SDS-PAGE was determined to be 12.4 kDa, and by HPLC, 25kDa was obtained. Therefore, the lectin is assumed to be composed of two identical subunits.

Blood and sugar specificity of the lectin - Human A, B, and O, and rabbit trypsinized blood cells were used for hemmaglutination activity. Only rabbit cells were agglutinated by the crude extract and purified preparation. Table 2 shows the hemagglutination inhibition of lectin by various sugars and glycoproteins. Simple sugars and disacchrides used were not inhibitory toward the hemagglutination. Yeast mannnan was not inhibitory at 1 mg/ml. Of the glycoproteins tested, quail ovomucoid and thyroglobulin were potent inhibitors. Asialofetuin was also inhibitory similarly for

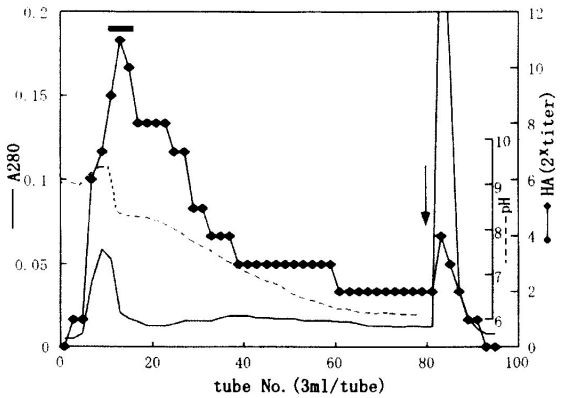

Figure 1. Chromatofocusing of *Arisaema* lectin after DEAE-Cellulofine chromatography. The basic fraction after DEAE-column chromatography was applied onto a PBE column. Solid line, absorbance at 280 nm; brocken line, pH; ◆, hemagglutination activity. Fractions with vertical bar were collected and used for further experiments. The arrow shows the start of elution with 2M NaCl.

Table 1. Summary for the purification of *Arisaema* lectin

Step	Total protein (mg)	Total activity (titer)	Specific activity (titer/mg/ml)	Yield (%)
Crude extract	2,064	2,000,000	960	100
$(NH_4)_2SO_4$	1,893	1,800,000	950	90
DEAE-Cellulofine (pH8.0)	5.5	86,000	16,000	4.3
Chromatofocusing	0.21	18,000	86,000	0.9

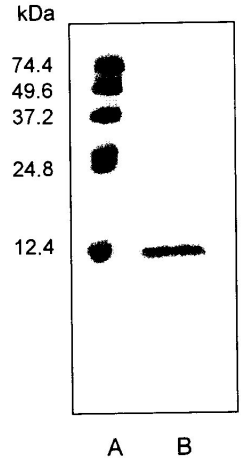

Figure 2. SDS-Electrophoresis. Fifiteen % gel was used for electrophoresis. Protein band was visualized with Coomasiee Brilliant Blue. Lane A, molecular-mass marker; Lane B, *Arisaema* lectin.

A. ringens lectin	NIPFTKNMDFAGQVL-
Arum 2*	NIPFTHNMLFSGQILY-
Colocasia 2*	NIPFTNNLLFSGQVXY-

Figure 3. N-terminal sequences of *Arisaema* lectin and two *Arum* lectins. Common amino acids are in the boxes. **Arum* 2 and *Colocasia* 2, ref [18].

Table 2. Inhibition of hemagglutinating activity of *Arisaema* lectin by sugars and glycoproteins Minimum concentration for complete inhibition of titer 4.

Inhibitor	A. *ringens* lectin	*Arum* lectin*
D-Mannose	No inhibition at 200 mM	100 mM
Ovalbumin	0.125	
Hen ovomucoid	0.25	
Quail ovomucoid	0.016	
Fetuin	0.125	0.2
Asialofetuin	0.031	0.013
Thyroglobulin	0.0078	0.1

*ref [18]. N-Acetyl D-glucosamine, L-fucose, D-galacose, D-glucosamine, D-glucose, lactose and methyl a-D-mannoside were not inhibitory at 200 mM. Man-α-1,3-Man and methyl α-Man-α-1,6-Man were not at 20 mM. Yeast mannan was not at 1mg/ml.

Arum lectin [18].

N-terminal amino acid sequencing - Figure 3 shows the comparison of N-teminal sequences of *A. ringens* lectin with those of two *Araceae* lectins. Of 15 amino acid residues, 9 amino acids were common in all the lectins.

Comments on the properties of the lectin - All the properties of *Arisaema* lectin described above are usually found in other *Araceae* lectins. However, the isoelectric point of *Arisaema* lectin, 8.2 was higher than those of other *Araceae* lectins [19].

Xanthosama lectin has two carbohydrate-binding sites for mannose and N-acetyllactosamine, respectively [3]. Although mannose and mannodisaccharides were not inhibitory toward hemagglutination activity of *Arisaema* lectin, glycoproteins with trimannosylcore or mannooligosaccharide such as quail ovomucoid and thyroglobulin were strong inhibitors. Furthermore, asialofetuin was also a potent inhibitor. The inhibitory potency of sugars toward *Arisaema*

lectin were different from the *Arum* lectin [18]. From the result of hemmaglutination inhibition, it is assumed that *Arisaema* lectin has two carbohydrate-binding sites. Structure-function relationship of mannose-bindng lectins is reported previously [20], but structural analysis of the binding site for N-acetyllactosamine has not been carried out for any *Araceae* lectins.

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