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Purification and properties of a β-galactosyl-specific lectin from the fruiting bodies of *Ischnoderma resinosus*

H. Kawagishi and T. Mizuno

Department of Agricultural Chemistry, Faculty of Agriculture, Shizuoka University, Ohya 836, Shizuoka 422, Japan

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A lectin was isolated from the fruiting bodies of *Ischnoderma resinosus* using affinity chromatography on Sepharose 4B. This lectin is composed of two identical subunits of 16 kDa. It exhibits specificity towards β-galactosyl residues.

Lectin; β-Galactosyl residue; Purification; Fruiting body; *(Ischnoderma resinosus)*

1. INTRODUCTION

Several lectins have been isolated from fruiting bodies of Basidiomycetes fungi; *Agaricus campestris* [1,2], *A. bisporus* [3,4], *A. edulis* [5], *Fomes formentarius* [6], *Clitocybe nebularis* [6], *Flammulina veltipes* [7], *Laccaria amethystina* [8] and *Volvariella volvacea* [9]. Among them, the lectins of three species have specificity toward monosaccharides; those of *F. formentarius* and *C. nebularis* are *N*-acetylgalactosamine specific, and two lectins of *L. amethystina* are *N*-acetylgalactosamine and L-fucose specific. The others do not exhibit specificity to any monosaccharides.

This paper reports on a β-galactosyl-specific lectin isolated from the fungus *Ischnoderma resinosus* which is known as a home remedy in Japan.

Correspondence address: H. Kawagishi, Department of Agricultural Chemistry, Faculty of Agriculture, Shizuoka University, Ohya 836, Shizuoka 422, Japan

Abbreviations: PBS, phosphate-buffered saline; IRA, *Ischnoderma resinosus* agglutinin; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; all sugars are of the D-configuration, unless otherwise stated

2. MATERIALS AND METHODS

2.1. *Isolation of* I. resinosus *agglutinin (IRA)*

All procedures were carried out at 4°C. Chipped fruiting bodies of *I. resinosus* (100 g) were extracted in 0.9% saline (2 l) with stirring. The resulting suspension was centrifuged (15 min, 10000 × *g*). Solid ammonium sulfate was added to the supernatant to give 80% saturation. The precipitate was collected by centrifugation (15 min, 20000 × *g*), resuspended in distilled water, dialyzed against distilled water and lyophilized. The crude extract was then applied to a Sepharose 4B column (10 ml) equilibrated with PBS. After extensive washing with PBS, the lectin was desorbed with 50 mM lactose in PBS. The eluates were concentrated, dialyzed against distilled water and lyophilized, giving a purified lectin.

2.2. *Assays and analytical methods*

Hemagglutination and hemagglutinating inhibition assay were performed according to Osawa et al. [4]. SDS-PAGE was performed as described by Laemmli [10]. Isoelectric focusing on a gel (LKB Ampholine Pagplate, pH 3.5–9.5) was performed in an LKB 2117 Multiphor apparatus with an LKB 2197 constant power supply. Carbohydrate analysis was carried out by the phenol-H_2SO_4 method with reference to glucose [11]. Amino acids were analyzed with a Hitachi model 835 amino acid analyzer after hydrolysis of samples in 6 M HCl at 110°C for 20 h in sealed evacuated tubes. Methionine and cysteine were estimated spectrophotometrically [13]. The N-terminal sequence was analyzed using an Applied Biosystems 470A automatic protein sequencer.

3. RESULTS AND DISCUSSION

The purification procedure is summarized in

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table 1. Since preliminary experiments indicated that crude extract exhibited galactosyl specificity, we chose Sepharose 4B as the affinity support.

The molecular mass of IRA estimated by gel filtration using Sephadex G-150 was about 32 kDa (not shown). Fig.1 shows SDS-PAGE of the purified lectin. Both reduced and unreduced (with and without 2-mercaptoethanol) lectin appeared as a single band of apparent molecular mass 16 kDa. These results allow us to conclude that the protein is composed of two identical subunits. Although size homogeneity was suggested by SDS-PAGE and gel filtration, isoelectric focusing over the pH range 3.5–9.5 indicated a family of bands focusing in the pH zone near 5 (fig.2). Amino acid sequence analysis gave no evidence of heterogeneity in the primary structure of the first 10 N-terminal residues (table 3). The multiple peaks observed in isoelectric focusing suggest that heterogeneity occurs in other regions of the molecule. Amino acids analysis revealed a high content of acidic and hydroxy amino acids, glycine, valine and leucine, and a low content of methionine, histidine and arginine (table 2). The carbohydrate content amounted to 4%.

As shown in table 4, IRA exhibited a slight preference for type B to type A and O erythrocytes. Pronase treatment increased the sensitivity of erythrocytes to agglutination by the lectin.

The carbohydrate-binding specificity of IRA is shown in table 5. Among monosaccharides, methyl β-galactoside is the most potent inhibitor. Fucose

Table 1

Purification of IRA (from 100 g fruiting bodies)

	Total protein (mg)	Total agglutination activity (titer)[a]	Specific agglutination activity (titer/mg protein)	Recovery (%)
80% $(NH_4)_2SO_4$ precipitate	1606	5780	3.6	100
Affinity chromatography	2.9	3719	1280	64

[a] Titer is defined as the reciprocal of the end-point dilution exhibiting the hemagglutination

Type A erythrocytes were used for testing

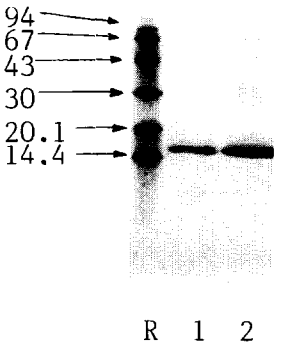

Fig.1. SDS-PAGE of IRA in the presence (lane 1) and absence (lane 2) of 2-mercaptoethanol. Lane R contains molecular mass (in kDa) marker proteins (phosphorylase *b*, albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor, α_1-lactalbumin).

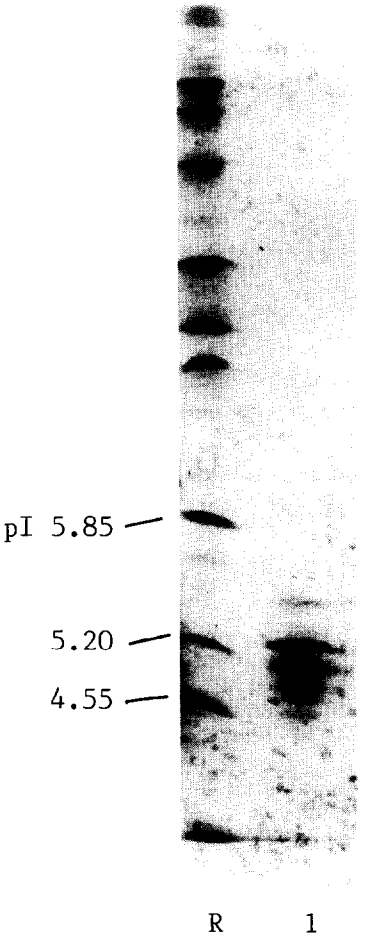

Fig.2. Isoelectric focusing of IRA (lane 1). Lane R contains p*I* marker proteins.

Table 2

Amino acid composition of IRA

Amino acid	mol%	Amino acid	mol%
Asx	10.4	Ile	2.8
Thr	9.9	Leu	8.9
Ser	5.7	Tyr	6.1
Glx	7.1	Phe	2.2
Gly	11.1	Lys	6.4
Ala	4.6	His	1.0
Val	12.1	Arg	1.5
Cys	2.2	Pro	4.5
Met	1.1	Trp	2.4

and L-arabinose are also inhibitory. Methyl α-galactoside is a weaker inhibitor than its β-anomer. Lactulose is the strongest inhibitor of all oligosaccharides tested. Lactose is the second most potent inhibitor. Both have β-galactosyl residues. Melibiose and raffinose, which contain α-galactosyl groups, are much weaker than the two β-galactosides. All other sugars tested were completely inert at concentrations up to 200 mM. These results suggest that IRA is clearly a galactosyl, in particular β-galactosyl, specific lectin. This lectin is also inhibited by fucose and L-arabinose. This can be explained in terms of the same configuration of C-2, -3 and -4 hydroxy groups to galactose. β-Galactosyl-specific lectins have been isolated from various plants [14,15], animals [16–18] and microorganisms [19]. Although at higher concentration, most are also inhibited by *N*-acetylgalactosamine. However, IRA is completely inert to it up to a concentration of 200 mM. These results indicate that this lectin strictly recognizes the C-2 hydroxy group in galactosyl residues. To our knowledge, this is the first reported isolation and characterization of a β-galactosyl-specific lectin from fungi and the second of a lectin from the order Polyporales [6].

Table 3

N-terminal sequence of IRA

Residue no.									
1	2	3	4	5	6	7	8	9	10
P	A	N	F	S	I	G	T	Y	A

Table 4

Agglutination profiles of IRA (100 μg/ml)

Group of erythrocytes	Untreated	Pronase-treated[a]
Human A	128	2048
Human B	256	4096
Human O	128	2048

[a] 10% suspension of erythrocytes in PBS (10 ml) was treated with pronase P (7 mg) for 30 min at 47°C

Table 5

Inhibition of hemagglutination of IRA by mono- and oligosaccharides

Inhibitor[a]	Minimum inhibitor concentration (mM)[b]	Specificity factor[c]
Methyl β-galactoside	0.60	1.0
Fucose	1.71	2.85
Galactose	3.13	5.22
L-Arabinose	5.60	9.33
Methyl α-galactoside	8.44	14.1
Lactulose[βGal(1→4)Fru]	0.20	0.33
Lactose[βGal(1→4)Glc]	1.06	1.77
Melibiose[αGal(1→6)Glc]	12.5	20.8
Raffinose[αGal(1→6)αGlc(1→2)βFru]	16.7	27.8

[a] Glucose, glucosamine, *N*-acetylglucosamine, galactosamine, *N*-acetylgalactosamine, mannose, mannosamine, fructose, xylose, rhamnose, ribose, deoxyribose, lactobionic acid, and maltose did not inhibit at all at concentrations up to 200 mM

[b] These are minimum concentrations required for inhibition of hemagglutination of IRA (titer 4)

[c] Calculated in relation to methyl β-galactoside

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