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A sialic acid-binding lectin from the mushroom *Hericium erinaceum*

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Abstract

A lectin was isolated from the mushroom *Hericium erinaceum*. This lectin is composed of two different subunits of 15 and 16 kDa and the molecular mass of the intact lectin was estimated to be 54 kDa by gel filtration. It exhibits specificity towards sialic acids, especially *N*-glycolylneuraminic acid.

Key words: Lectin; sialic acid-binding; Purification; Mushroom; Fungus; *Hericium erinaceum*

1. Introduction

Sialic acid-specific lectins have been isolated from various animals, plants, and microorganism [1–17]. However, lectins having such binding specificity isolated from fungi have not been reported.

Hericium erinaceum is an edible mushroom from which we have isolated many bioactive substances [18–21]. During our continuing research into bioactive substances from this mushroom, we found the lectin activity showing sialic acid-specificity in the mushroom. This paper reports on the sialic acid-binding lectin isolated from the mushroom which known as a Chinese medicine.

2. Materials and methods

2.1. Isolation of HEL

All the procedures were carried out at 4°C. Fresh fruiting bodies of *H. erinaceum* (100 g) were homogenized with acetone (500 ml) in a blender, and extracted overnight. The resulting suspension was filtered with filter paper under reduced pressure and the residues was then extracted with saline overnight. The resulting suspension was filtered with gauze and the filtrate was centrifuged (15 min, 10,000 × *g*). The supernatant was fractionated with 30–80% saturated ammonium sulfate. The precipitate collected by centrifugation (15 min, 10,000 × *g*) was dialyzed against distilled water, and lyophilized. The lyophilized material (72.8 mg) was redissolved in 20 mM Tris-glycine buffer (pH 7.5) and applied to a DEAE-Toyopearl column equilibrated with the buffer. After washing with the same buffer, the adsorbed materials were eluted stepwise with 0.05, 0.1, 0.2, and then 1 M NaCl in the buffer. Lectin-containing fraction (the eluate with 0.2 M NaCl) was dialyzed against 50 mM acetate buffer (pH 4.5) and further applied to a Mono-S column with FPLC system. Purified lectin was obtained by elution from the column with a linear gradient of 0–1 M NaCl in the acetate buffer followed by dialysis against distilled water and then liophilyzation.

2.2. Assays and analytical methods

Hemagglutination and hemagglutination inhibition tests, SDS-PAGE, isoelectric focusing, pH stability, thermostability, effect of metal cations on the lectin activity, amino acid composition, and N-terminal amino acid of the protein were examined as described previously [22–25]. Sialidase-treatment was done by the method of Mercy and Ravindranath [16]. Sugar was measured by the phenol–H_2SO_4 method with reference to glucose [26].

3. Results and discussion

The results of the purification are summarized in Table 1. The ammonium sulfate-precipitate was applied to a DEAE-Toyopearl column and all the lectin activity was adsorbed to the column. The activity was eluted with 0.2 M NaCl in Tris buffer. The fraction was further purified by FPLC with a Mono-S column. From 100 g of the fruiting bodies, 1.0 mg of the purified lectin, which was named HEL, was obtained. Since the hemagglutination by the lectin was inhibited by BSM and asialo-BSM in the hemagglutination-inhibition test (Table 2), the lectin could be also purified from the ammonium sulfate precipitates by affinity chromatography on BSM- or asialo-BSM-Toyopearl. But, recovery of the activity by the affinity chromatography was much lower (10% and 8.7%, respectively) than that of the former procedure.

HEL gave two bands which were very close each other on SDS-PAGE regardless of presence or absence of 2-mercaptoethanol and the molecular mass of the two subunits were 15 kDa and 16 kDa (Fig. 1). The molecular mass of the intact lectin was estimated to be 54 kDa by

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Abbreviations: BSM, bovine submaxillary mucin; HEL, *Hericium erinaceum* lectin; NeuAc, *N*-acetylneuraminic acid; NeuGc, *N*-glycolylneuraminic acid; PBS, 10 mM phosphated-buffered saline (pH 7.4), SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis; all sugars are of D-configuration, unless otherwise stated.

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Table 1
Purification of HEL (from 100 g of fruiting bodies)

Fraction	Total protein (mg)	Total agglutination activity (titer)[a]	Specific agglutination activity (titer/mg protein)	Recovery of activity (%)[b]
30–80% $(NH_4)_2SO_4$ precipitate	72.8	14,560	200	100
Eluate from DEAE-Toyoperal	10.0	8,270	827	56.8
Eluate from Mono-S HR5/5	1.0	6,360	6,360	43.7

[a] Titer defined as the reciprocal of the end-point dilution exhibiting the hemagglutination with pronase-treated type A erythrocytes in PBS.
[b] Based on the initial 30–80% $(NH_4)_2SO_4$ precipitate.

gel filtration on Superose-12 (not shown). Since the lectin purified by the affinity chromatographies also gave the same results, we concluded that the protein obtained by the two ion-exchange chromatographies on DEAE-Toyopearl and Mono-S was a purified lectin. Thus, the lectin appears to be a tetramer containing two different subunits.

The value of the carbohydrate content was 1.5%. Amino acid analysis revealed a high content of Gly, Glx, Ser, Ala and a low content of Tyr, His, Met (Table 3). Although 3 nmol of the protein was applied to the sequencer, N-terminal amino acid could not be detected, suggesting that N-terminus of HEL was probably blocked.

The isolelectric focusing gave a family of bands in pH zone near 5.0 (not shown). This lectin was quite stable between pH 5.0 and 10.5, and below 70°C (not shown). The hemagglutinating activity of the lectin was not affected by demetalization with EDTA, and addition of $CaCl_2$, $MgCl_2$, $ZnCl_2$ or $MnCl_2$ to the demetalized lectin did not cause any change of the activity.

As indicated in Table 4, HEL exhibited a slight preference for type A and O to type B erythrocytes. Pig erythrocytes which was rich in NeuGc were more effective to the lectin than human ones. Pronase- and sialidase-treatment increased the sensitivity of erythrocytes to agglutination by the lectin.

The carbohydrate-binding specificity of HEL was shown in Table 2. Many monosaccharides and oligosaccharides were tested. However, only 4 monosaccharides and a oligosaccharide could inhibit the lectin-mediated hemagglutination. Among them, NeuGc was the most potent inhibitor and NeuAc was the next one. The minimum concentration of NeuGc required for inhibition of HEL-induced hemagglutination was 4 times greater than that of NeuAc. 3′-*N*-Acetylneuramin-lactose and galacturonic acid were also inhibitory at higher concentration. Since the other acidic saccharides such as glucuronic acid, glucose-6-phosphate, glucose-1-phosphate, AMP, ADP, ATP, or lactobionic acid did not inhibit the hemagglutination, the inhibition by the sialic acids was not due to the acidity of the sugars and non-specific interaction between the protein and the carboxyl groups in the sugars. Among glycoproteins tested, asialo-BSM

Table 2
Inhibition of the hemagglutinating activity of HEL by mono- and oligo-saccharides and glycoproteins

Inhibitor[a]	Minimum inhibitory concentration[b]	Specificity factor[c]
	(mM)	
N-glycolylneuramic acid	1.56	1.00
N-acetylneuramic acid	6.25	4.00
3′-*N*-acetylneuramin-lactose	12.5	8.00
galacturonic acid	25.0	16.0
	(μg/ml)	
asialo-BSM	0.006	
asialo-fetuin	0.39	
BSM	0.78	
fetuin	6.25	
α_1-acid glycoprotein	15.6	

[a] D,L-Arabinose, D,L-fucose, fructose, xylose, glucose, glucosamine, *N*-acetylglucoasamine, galactose, galactosamine, *N*-acetylgalactosamine, *N*-acetylmannosamine, mannosamine, mannose, ribose, 2-deoxyribose, glucuronic acid, glucose-6-phosphate, glucose-1-phosphate, AMP, ADP, ATP, lactose, lactulose, lactobionic acid, melibiose, maltose, sucrose, *N*-acetylchitobiose, and chitobiose exhibited no inhibition at concentrations up to 0.2 M.
[b] Minimum concentrations required for inhibition of 4 hemagglutinating doses of HEL.
[c] Calculated in relation to NeuGc.

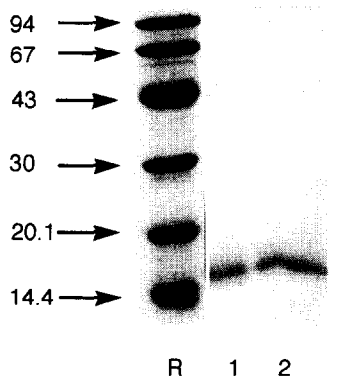

Fig. 1. SDS-PAGE of HEL in the presence (lane 1) and absence (lane 2) of 2-mercaptoethanol. Lane R contains molecular mass (in kDa) marker proteins (phosphorylase b, albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor, α-lactalbumin).

Table 3
Amino acid composition of HEL

Amino acid	mol%	Amino acid	mol%
Asx	7.2	Ile	5.9
Thr	4.8	Leu	4.1
Ser	9.1	Tyr	2.5
Glx	10.1	Phe	3.9
Gly	16.1	Lys	6.5
Ala	6.1	His	2.7
Val	7.7	Arg	3.9
Cys	n.d.[a]	Pro	3.5
Met	3.1	Trp	n.d.

[a] n.d. = not determined.

was the best inhibitor and the inhibitory activity of the glycoprotein was much stronger than that of native BSM. Asialofetuin was also more inhibitory than native fetuin. There are some discrepancies among the results of mono- and oligo-saccharide-specificity, glycoprotein-specificity, and sialidase-treatment of erythrocytes. This lectin may be able to recognize free sialic acids more strongly than those acids in sugar chains. In addition, the binding of HEL to the asialo-glycoproteins and the sialidase-treatment erythrocytes is not due to only simple interaction between the lectin and the sugar chains of the asialo-glycoproteins, since trypsin- or pronase-digested asialo-BSM or asialofetuin showed much weaker inhibitory effect on HEL-mediated hemagglutination than native one (not shown). To our knowledge, this is the first reported isolation and characterization of a sialic acid-binding lectin from fungi. *Hericium erinaceum* is one of wood-rotting fungi. There are no previous reports of isolation or detection of sialic acids from fungi or plants. Although many lectins have been isolated from mushrooms, the biological roles of the mushrooms are still unknown [22–25]. It is important and interesting to know the 'real' binding substance to HEL. We found that the specific binding substances to HEL were contained in the mushroom itself and succeeded in partial purification of the substances (not shown). Further work along this line is in progress.

Table 4
Agglutination profiles of HEL (100 μg/ml)

Group of erythrocytes	Untreated	Pronase-treated[a]	Sialidase-treated[b]
Human A	32	1,024	4,096
Human B	16	512	1,024
Human O	32	1,024	2,048
Pig	64	2,048	2,048

[a] 10% suspension of erythocytes in PBS (10 ml) was treated with pronase P (7.0 mg) for 30 min at 47°C.
[b] According to the method of Mercy and Ravindranath [16].

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