Carbohydrate Research, 183 (1988) 150–154
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Isolation and properties of a lectin from the fruiting bodies of *Agaricus blazei*

HIROKAZU KAWAGISHI*, AYA NOMURA, TAKAYUKI YUMEN, TAKASHI MIZUNO,
Department of Agricultural Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Shizuoka 422 (Japan)

TOSHIHIKO HAGIWARA, AND TAKUJI NAKAMURA
Nichirei Research Institute, Tokyo 189 (Japan)

(Received February 8th, 1988; accepted for publication, in revised form, May 18th, 1988)

Several lectins have been isolated from the fruiting bodies of basidiomycetous fungi. Among the species studied are *Agaricus campestris*[1,2], *Agaricus bisporus*[3,4], *Agaricus edulis*[5], *Fomes fomentarius*[6], *Clitocybe nebularis*[6], *Flammulina veltipes*[7], *Laccaria amethystina*[8], and *Volvariella volvacea*[9]. Recently, we have also isolated a β-D-galactosyl-specific lectin (IRA) from *Ischnoderma resinosum*[10]. The present paper reports the isolation and some properties of a lectin from *A. blazei* (Japanese name: Himematsutake), and comparison of this lectin with the other *Agaricus* lectins.

EXPERIMENTAL**

Hemagglutination and hemagglutination-inhibition assays were performed according to the methods of Osawa *et al.*[4] and Vasta *et al.*[11], using type A erythrocytes. SDS-PAGE** was performed according to the method of Laemmli[12]. Carbohydrate analysis was carried out by the phenol–H_2SO_4 method[13], with D-glucose as the reference standard. Amino acids were analyzed with a Hitachi Model 835 amino acid analyzer after hydrolysis of samples in 6M HCl for 20 h at 110° in sealed, evacuated tubes. Methionine and cysteine were determined after peroxyformic acid oxidation[14]. Tryptophan was estimated spectrophotometrically[15]. The temperature-stability of the lectin was estimated by the method of Vasta and Marchalonis[16].

All procedures were carried out at 4°. The fresh fruiting-bodies (1 kg) were

*To whom correspondence should be addressed.

**Abbreviations: PBS, phosphate-buffered saline (pH 7.4); SDS-PAGE, sodium dodecyl sulfate–poly(acrylamide) gel electrophoresis; BSM, bovine submaxillary mucin. All sugars were of the D configuration, unless stated otherwise.

0008-6215/88/$ 03.50

homogenized in a blender with 0.9% saline (3 L). The mixture was stirred overnight, and then centrifuged at 10,000*g* for 15 min. The supernatant liquor was heated for 2 h at 60°. The precipitate collected between 30 and 80% saturation of ammonium sulfate was dialyzed, first against tap water and then de-ionized water, and lyophilized. The lyophilized material was redissolved in PBS, and applied to a column (3.0 × 40 cm) of DEAE-Toyopearl 650M equilibrated with PBS. All colored material was adsorbed to the column, and the hemagglutinating activity was recovered in the unadsorbed fraction. The adsorbed materials were eluted stepwise with phosphate buffer (pH 7.4) containing 0.2M, 0.5M, and then M NaCl. The colorless effluent was concentrated by ultrafiltration, and then applied to a column (1.5 × 5 cm)[11] of asialofetuin–Sepharose 4B equilibrated with PBS. After extensive washing with PBS, this adsorbent was transferred to a centrifuge tube with 0.2M NH_4OH (10 mL), and the lectin was desorbed by ultrasonication (41 kHz, 35 W) for 3 min. After centrifugation at 1,000*g*, the supernatant liquor was concentrated by ultrafiltration, dialyzed against distilled water, and lyophilized, giving the purified lectin.

RESULTS AND DISCUSSION

The results of the purification are summarized in Table I. The ammonium sulfate precipitates were applied to a column of DEAE-Toyopearl, to remove dark-brown material. Some 64% of the hemagglutinating activity came through unretarded, and no more activity was eluted with NaCl at concentrations up to M. Elution of the lectin from the column of asialofetuin–Sepharose 4B was attempted with 0.2M NH_4OH, M NaCl, 3M KSCN, 0.1M AcOH, 0.1M glycine·HCl (pH 3.0), 0.2M *N*-acetyl-D-galactosamine, and methyl *N*-acetyl-α-D-galactosaminide. Among these reagents, 0.2M NH_4OH gave the best results, but the actual recovery was low (9.2% of the activity precipitated by ammonium sulfate). After various attempts to increase the recovery, we found that elution by ultrasonication in 0.2M NH_4OH was effective. This method improved the recovery of the lectin to 16%.

TABLE I

PURIFICATION OF *Agaricus blazei* LECTIN FROM 1 kg OF THE FRUITING BODIES

Fraction	*Protein concentration (mg/mL)*	*Total protein (mg)*	*Titer*[a]	*Total activity (titer/mL)*	*Specific activity (titer/mg/mL)*	*Recovery (%)*[b]
30–80% $(NH_4)_2SO_4$ precipitate	1.33	3150	32	75600	24	100
DEAE-Toyopearl effluent	1.35	509	128	48400	95	64
Eluate from asialofetuin–Sepharose 4B by ultrasonication	1.44	8.7	2048	12400	1420	16
by percolation	1.44	4.9	2048	6960	1420	9.2

[a]Titer defined as the reciprocal of the end-point dilution exhibiting the hemagglutination. [b]Based on the initial $(NH_4)_2SO_4$ precipitate.

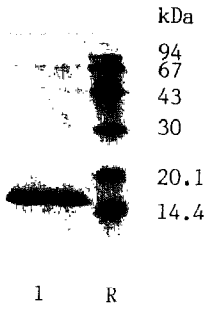

Fig. 1. SDS-PAGE of *Agaricus blazei* lectin (lane 1). Lane R contains the marker proteins (from top, phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and α-lactalbumin).

The molecular weight of the lectin, estimated by gel filtration on Toyopearl HW-55S was 60,000–70,000 (data not shown). SDS-PAGE revealed that molecular weight of the subunits was ~16,000 (see Fig. 1). These results allowed us to conclude that the protein is composed of four identical subunits. Amino acid analysis revealed a high content of acidic and hydroxy amino acids and glycine, and a low content of sulfur-containing and aromatic amino acids (see Table II). The value for carbohydrate content was 11%. The lectin was completely stable at temperatures below 65° (see Fig. 2).

The *A. blazei* lectin agglutinated all types of human erythrocytes equally. Table III shows the results of hemagglutination-inhibition assays. Among the glycoproteins, asialo-BSM and BSM, which have glycosidically linked sugar chains, and asialofetuin and fetuin, which have both glycosidically and *N*-glycosylically linked chains, inhibited the hemagglutination. Human serum transferrin, which contains only *N*-glycosylically linked sugar chains, had no effect on the hemagglutination. Furthermore, desialylated BSM and fetuin were more effective than the native

TABLE II

AMINO ACID COMPOSITION OF *Agaricus blazei* LECTIN

Amino acid	*mol%*	*Amino acid*	*mol%*
Asx	11.1	Ile	3.3
Thr	6.8	Leu	6.7
Ser	11.3	Tyr	1.7
Glx	11.8	Phe	3.5
Gly	13.3	Lys	2.8
Ala	4.8	His	2.5
Val	6.1	Arg	3.2
Cys	1.3	Pro	7.4
Met	1.1	Trp	1.3
			100.0

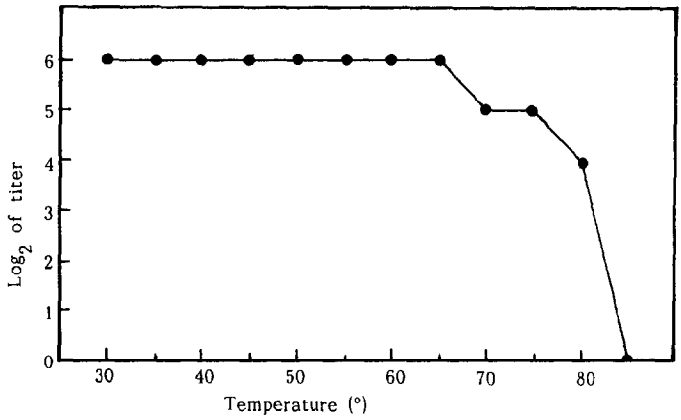

Fig. 2. Thermostability of *Agaricus blazei* lectin. Samples (45 μg/mL) in PBS were heated for 30 min at the temperatures indicated, cooled in ice, and titrated.

materials. Also, hemagglutination by *A. bisporus* agglutinins (named ABA I–IV) was inhibited by BSM, and not inhibited by human serum transferrin[4]. Among various simple sugars, D-glucose, D-galactose, D-mannose, D-fructose, D-fucose, L-fucose, L-rhamnose, D-arabinose, L-arabinose, D-xylose, D-galactosamine, D-glucosamine, D-mannosamine, *N*-acetyl-D-glucosamine, methyl *N*-acetyl-β-D-galactosaminide, methyl *N*-acetyl-D-glucosaminide (both anomers), raffinose, melibiose, lactose, lactulose, lactobionic acid, and *N*-acetyllactosamine did not inhibit, at all, at concentrations up to 200mM. *N*-Acetyl-D-galactosamine (2-acetamido-2-deoxy-D-galactose) and its methyl α-glycoside inhibited the hemagglutination at high concentrations. On the other hand, hemagglutination by

TABLE III

INHIBITION OF THE HEMAGGLUTINATING ACTIVITY OF *Agaricus blazei* LECTIN BY GLYCOPROTEINS AND MONOSACCHARIDES

Inhibitors	*Minimum inhibitory concentration*[a]
	(μg/mL)
Asialo-BSM	0.0098
BSM	0.60
Asialofetuin (bovine)	0.87
Fetuin (bovine)	31
Transferrin (human)	
	*m*M
Methyl *N*-acetyl-α-D-galactosaminide	100
N-Acetyl-D-galactosamine	200

[a]These are minimum concentrations required for inhibition of 4 hemagglutinating doses of the lectin.

TABLE IV

SOME PROPERTIES OF THE LECTINS FROM *Agaricus* MUSHROOMS

Lectins	*Human blood-type specificity*	*Mol. wt.*	*Subunits per molecule*	*Carbohydrate content (%)*
A. blazei	no	64,000	4	11
A. bisporus[3]	no	64,000	4	4
A. campestris[1,2]	no	64,000	4	4
A. edulis I[5]	no	60,000	4	18
A. edulis II[5]	no	32,000	2	2

ABA's was inhibited only by methyl *N*-acetyl-α-D-galactosaminide at[4] 200mM, and that by *A. edulis* lectin was not inhibited by any simple sugar tested[5].

Some properties of the lectins from *Agaricus* mushrooms are summarized in Table IV. These lectins are similar to each other. However, *A. blazei* lectin is different from the other *Agaricus* lectins in its amino acid composition and carbohydrate-binding specificity[1–5].

ACKNOWLEDGMENT

We thank the Iwade Institute of Mushrooms for a gift of the fruiting bodies of *A. blazei*.

REFERENCES

1 H. J. SAGE AND J. J. VAZQUEZ, *J. Biol. Chem.*, 242 (1967) 120–125.
2 H. J. SAGE AND S. L. CONNETT, *J. Biol. Chem.*, 244 (1969) 4713–4719.
3 C. A. PRESANT AND S. KORNFELD, *J. Biol. Chem.*, 247 (1972) 6937–6945.
4 S. SUEYOSHI, T. TSUJI, AND T. OSAWA, *Hoppe-Seyler's Z. Physiol. Chem.*, 366 (1985) 213–221.
5 R. EIFLER AND P. ZISKA, *Experientia*, 36 (1980) 1285–1986.
6 V. HOŘEJŠÍ AND J. KOCOUREK, *Biochim. Biophys. Acta*, 538 (1978) 299–315.
7 M. TSUDA, *J. Biochem. (Tokyo)*, 86 (1979) 1463–1468.
8 J. GUILLOT, L. GENAUD, J. GUEGNOT, AND M. DAMEZ, *Biochemistry*, 22 (1983) 5365–5369.
9 J. LIN AND T. CHOU, *J. Biochem. (Tokyo)*, 96 (1984) 35–40.
10 H. KAWAGISHI AND T. MIZUNO, *FEBS Lett.*, 227 (1988) 99–102.
11 G. R. VASTA, J. C. HUNT, J. J. MARCHALONIS, AND W. W. FISH, *J. Biol. Chem.*, 261 (1986) 9174–9181.
12 U. K. LAEMMLI, *Nature (London)*, 227 (1970) 680–685.
13 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 280 (1956) 350–356.
14 S. MOORE, *J. Biol. Chem.*, 238 (1963) 235–237.
15 H. EDELHOCH, *Biochemistry*, 6 (1967) 1948–1954.
16 G. R. VASTA AND J. J. MARCHALONIS, *J. Biol. Chem.*, 261 (1986) 9182–9186.