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A Lectin with Mycelia Differentiation and Antiphytovirus Activities from the Edible Mushroom *Agrocybe aegerita*

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A lectin named AAL has been purified from the fruiting bodies of the edible mushroom *Agrocybe aegerita*. AAL consisted of two identical subunits of 15.8 kDa, its pI was about 3.8 determined by isoelectric focusing, and no carbohydrate was discerned. Being treated by pyrogultamate aminopeptidase, the blocked N-terminus of AAL was sequenced as QGVNIYNI. AAL agglutinated human and animal erythrocytes regardless of blood type or animal species. Its hemagglutinating activity was unaffected by acid or alkali treatment and demetalization or addition of divalent metals Mg^{2+}, Ca^{2+} and Zn^{2+}. AAL was toxic to mice: its LD50 was 15.85 mg per kilogram body weight by intraperitoneal injection. In this study, two novel activities of AAL were proved. It showed inhibition activity to infection of tobacco mosaic virus on *Nicotiana glutinosa.* The result of IEF suggested that AAL attached to TMV particles. Mycelia differentiation promotion was the other interesting activity. AAL promoted the differentiation of fruit body primordia from the mycelia of *Agrocybe aegerita* and *Auricularia polytricha*. AAL antiserum was prepared and immunologically cross-reactived with several proteins from five other kinds of mushrooms. These results suggested that AAL probably was a representative of a large protein family, which plays important physiological roles in mushroom.

Keywords: *Agrocybe aegerita*, Lectin, TMV inhibitor, Differentiation promotion

Introduction

Lectins are proteins/glycoproteins that have been found in various organisms (Peumans *et al*., 1995). They have been isolated from a diversity of plants, animals, and microorganisms. They play many important roles in their growth processes. Most plant lectins are storage proteins, which acquire a potential role in defence when the plant or the seed is confronted by insects and fungi (Gatehouse *et al*., 1995; Peumans *et al*., 1995). Some legume lectins mediate the symbiotic association between leguminous plants and nitrogen-fixing bacteria (Brock *et al*., 1991). β-Galactoside-specific animal lectins regulate differentiation and organ formation (Sharon *et al*., 1983).

In 1994, Pemberton tested 403 species of fungi, and proved that half of them contained hemagglutinins (Pemberton, 1994). About 50 mushroom lectins have been purified and recently been reviewed (Guillot and Konska, 1997; Wang *et al*., 1998). They are heterogeneous assembly of proteins with a wide range of molecular weights, carbohydrate contents, and amino acid sequences. They are currently attracting much interest on account of their many exploitable medicinal activities. The immunomodulatory and antitumor/cytotoxic activities of lectins from *Agaricus bisporus* (Yu *et al*., 1993, 1999) *Boletus satanas* (Ennamany *et al*., 1994), *Flammulina velutipes* (Hsu *et al*., 1997), *Ganoderma lucidum* (Van *et al*., 1995), *Grifola frondosa* (Kawagishi *et al*., 1990), *Tricholoma mongolium* (Wang *et al*., 1995) and *Volvariell volvacea* (Lin *et al*., 1984) have been testified. A collagen-binding protein, which inhibits the lewis lung carcinoma cell adhesion to type IV collagen was isolated from *Hypsizigus marmoreus* (Tsuchida *et al*., 1995).

Compared with many reports of medicinal activities, few studies on physiological activities of mushroom lectins, which had been made many investigations on plant lectins and animal lectins, were carried out. Although Wang (1998) deduced in his review that mushroom lectins probably play an important role in dormancy, growth, and morphogenesis, morphological changes consequent on parasitic infection, no direct evidence was given except *Lactarius deterrimus* lectin as a molecular recognition during the early stages of mycorrhization (Giollant *et al*., 1993).

In this study, we isolated and characterized a *Agrocybe*

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aegerita lectin (AAL) with inhibitory activity on tobacco mosaic virus (TMV) and differentiation promotion activity of the fruit body promodia from the mycelia. These activities were herein the first report.

Materials and Methods

Materials The dry fruiting bodies of *Agrocybe aegerita* were obtained from Sanming institute of fungi (R. P. China). DEAE-Sepharose F.F., Sephacryl-200 HR, and Superdex 75HR were purchased from Pharmacia (Uppsala, Sweden). Bovine submaxillary mucin (BSM), glycophorin A, κ-Casein, hog gastric mucin, chicken ovomucoid, and porcine thyroglobulin for the hemagglutinating inhibition tests were kindly supplied by professor Zeng Z. K, Sichuan University, Chengdu, China. Asialoglycoproteins were prepared by hydrolysis with 0.1 M H_2SO_4 at 80ºC. All the other sugars for the test were products of Sigma (St. Louis, USA).

Isolation of AAL Dry fruiting bodies were crushed into powder. 15 g powder was extracted twice by 150 ml distilled water at 4ºC for 10 h. The extracts were combined and centrifuged at 10,000 × *g* for 20 min. The supernatant was added solid ammonium sulfate to get 40% saturation and centrifuged again. Then solid ammonium sulfate was added into the supernatant to get 80% saturation, and the 40-80% precipitate was collected by centrifugation, dissolved in a small volume of distilled water and dialyzed extensively against 10 mM phosphate buffer (pH 6.0). The dialyzate was put on a column (1.6 × 10 cm) of DEAE-Sepharose column that had been pre-equilibrated with the same buffer. The column was eluted with a linearly increasing concentration of NaCl from 0-0.3 $mol \cdot l^{-1}$ in the starting buffer at a flow rate of 60 $ml \cdot h^{-1}$. Then three elution protein peaks were obtained. The samples contained the second peak were pooled and applied on a Sephcryl-200 (1.6 × 80 cm) column that was eluted with 0.025 M PB (pH 7.1) at a flow rate of 32 $ml \cdot h^{-1}$. A big protein peak appeared in the chromatographic profile. This peak was collected and lyophilized, giving the purified lectin.

Characterization of AAL The molecular mass of AAL was determined by both SDS-PAGE and gel filtration. SDS-polyacrylamide gel electrophoresis was performed by using a discontinuous system with a 12% acrylamide running gel and 5% stacking gel. Protein bands were stained with coomassie brillant blue R-250. Gel filtration for measuring the molecular weight of native lectin was carried out on a Superdex 75 HR column. Standard protein markers used included cytochrome C (MW 12.4 kDa), chymotrypsinogen A (MW 25 kDa) and ovalbumin (MW 45 kDa). The isoelectric point was determined on a 5% polyacrylamide gel containing 2% ampholine selected to establish a pH gradient from 3.5 to 9.5. The phenol-sulfuric acid method was used to examine the possible presence of a carbohydrate moiety of the lectin.

Amino acid composition and N-terminal amino acid sequence Amino acid analysis was done in a Hitachi model 835 analyzer. Samples (50 µg) were hydrolyzed in 6 M HCl in sealed evacuated tubes for 24 h at 110ºC. The N-terminal amino acid residues were analyzed using an Applied Biosystems 470A automatic protein sequencer.

Hemagglutinating assays Erythrocytes of 12 various animals and human A, B, O type were tested independently in this assay. The animals were housed under normal laboratory conditions (21 ± 2ºC, 12/12 h linght/dark cycle). A serial two-fold dilution of the lectin solution in microtiter U-plates (50 µl) was mixed with 50 µl 2% suspension of red blood cells in phosphate buffered saline (pH 7.2) at 20ºC. The results were read after about 1h when the control (no lectin was added) had fully precipitated. The hemagglutination titer, defined as the reciprocal of the highest dilution exhibiting hemagglutination, was reckoned as one hemagglutination unit (2^n). Specific activity is the number of hemagglutination units per mg protein.

The hemagglutinating inhibition tests To investigate inhibition of lectin-induced hemagglutination by various carbohydrates hemagglutinating inhibition tests were performed in a manner analogous to the hemagglutination test. Serial two fold dilutions of sugar samples were prepared in phosphate buffered saline. All of the dilutions were mixed with an equal volume (25 µl) of a AAL solution of with 32 (2^5) hemagglutination units. The mixture was allowed to incubate for 30 min at room temperature and then mixed with 50 µl of a 2% rabbit erythrocyte suspension. The minimum concentration of the sugar in the final reaction mixture that completely inhibited 32 hemagglutination units of the AAL preparation was calculated.

Stability assays For testing the thermal-stability, AAL solutions of the same concentration (1 mg/ml) were preincubated for 10 min at different temperatures in a thermostatic bath. The hemagglutination activity of each of the treated lectins was then determined as described above.

The pH stability of the lectin was measured by incubating the samples in 0.03 N, 0.06 N, 0.12 N and 0.24 N HCl or NaOH. After 30 min an equal volume of NaOH or HCl was added to get a neutral solution. The hemagglutination activity of the acid- or alkali-treated lectins was determined. The control groups were treated with phosphate buffered saline (PBS, pH 7.2).

Effects of metal cations on the lectin activity To examine the cationic requirements of hemagglutination induced by the lectin, the samples was demetalized by dialysis exhaustively against 10 mM EDTA and the hemagglutination activity of the demetalized lectin was determined. The treated lectin was then incubated with one of the following cations: 1 mM $CaCl_2$, $MgCl_2$, $ZnCl_2$ in PBS (pH 7.2) for 1 h, and its hemagglutination activity was determined again.

Lethality The lethality of lectin was determined according to the method of Lin (1984).

Anti-phytoviral activity test Tobacco mosaic virus-ordinary strain (TMV) was multiplied in tobacco plants (*Nicotiana tabacum* L. Var. Xanthi). TMV was purified according to the method of Veldee *et al.* (1962). *Nicotiana glutinosa* was grown in a greenhouse and used as a local lesion host for TMV. The inhibitory

activity of plant virus infection was assayed according to the method of Gianinazzi and Kassanis (1974). AAL solution in 0.01 M PBS buffer (pH 7.2) was mixed with equal volume of a TMV solution (10 μg/ml) in the same buffer. This mixture containing carborundum was inoculated using a gum sponge into the intercellur spaces in one half of each of the six leaves. The other half-leaves were inoculated with the same virus solution without AAL as a control. The leaves were then rapidly washed with pure water. The relative inhibition ratio (%) of the inhibitor was calculated according to the following equation:

Inhibition ratio (%) = (1 − number of lesions by the sample/ number of lesions by the control) × 100%

To confirm the interaction between AAL and TMV, AAL and TMV were incubated at room temperature for 30 min and run on a isoelectric focusing gel with TMV and AAL accompanied.

Antifungal activity test At the center of 9 cm petri dishes containing 10 ml potato dextrose agar was inoculated the tested fungal mycelium. When the fungus grows to a diameter of 3 cm at 28°C around the fungal colony and at a distance of 1 cm from it were placed sterile blank paper disks of 0.5 cm. A 10 μl AAL of serial concentration in 10 mmol/L phosphate buffer (pH 7.2) was added to a disk, 10 μl of the buffer served as control. Three plant pathogenic fungi: *Trichoderma viride*, *Colletotrichum musae*, and *Fusarium oxysporum*, were examined in the assay.

Differentiation promotion to mycelia The mycelia of two mushrooms (*Agrocybe aegerita* and *Auricularia polytricha*) were inoculated in the center of two separated plates containing potato dextrose agar. The plates were incubated at 28°C in darkness. After the mycelia covered the plate (about 7 days), AAL of different concentrations were dripped on the mycelia around at a distance 3 cm from center. Then the plates growing mycelia of *Agrocybe aegerita* and *Auricularia polytricha* were incubated in light at the temperatures of 20°C and 15°C respectively. About one month later, the formation of fruiting bodies could be observed. The air relative humidity was not less than 85% through all the protocol.

Immunochemical procedures For preparation of lectin antiserum, a rabbit was immunized by intramuscular injection of pure lectin mixed with an equal volume of freund's incomplete adjuvant. For immunoblotting, crude protein solutions were prepared from dry fruiting bodies of 5 mushrooms, including *Hericium ramosum, Pleurotus erygii, lentinula edodes, volvariella volvacea,* and *Ganoderma Lucidum* by buffer extraction and ammonium sulfate precipitated at 80% saturation. Proteins were electrophoretically transferred from the gels to PVDF membranes at 1 mA/cm^2 for 2 h at 4°C. The membranes were blocked by 3% skim milk overnight, and the homogenous proteins were visualized by the treatment with anti-lectin serum coupled to a anti-rabbit antibody labeled with alkaline phosphatase.

Results

Purification of lectins The purification protocol of AAL is shown in Fig. 1. The crude protein prepared from ammonium sulfate saturation (40-80% saturation) was applied on a DEAE-Sepharose column (1.6 × 10 cm). Three peaks D1, D2, and D3 were observed by 200 ml 0-0.3 mol/L NaCl gradient elution. The D2 peak corresponded to 0.15 N NaCl. The fractions containing peak D2 were pooled and concentrated. This sample was applied on a Sephacyl-200 column. The fractions corresponding to the main peak D2S1 were collected and represented purified lectin. In a typical purification experiment, about 0.35 mg purified AAL was obtained from 1 g dry fruiting body powder.

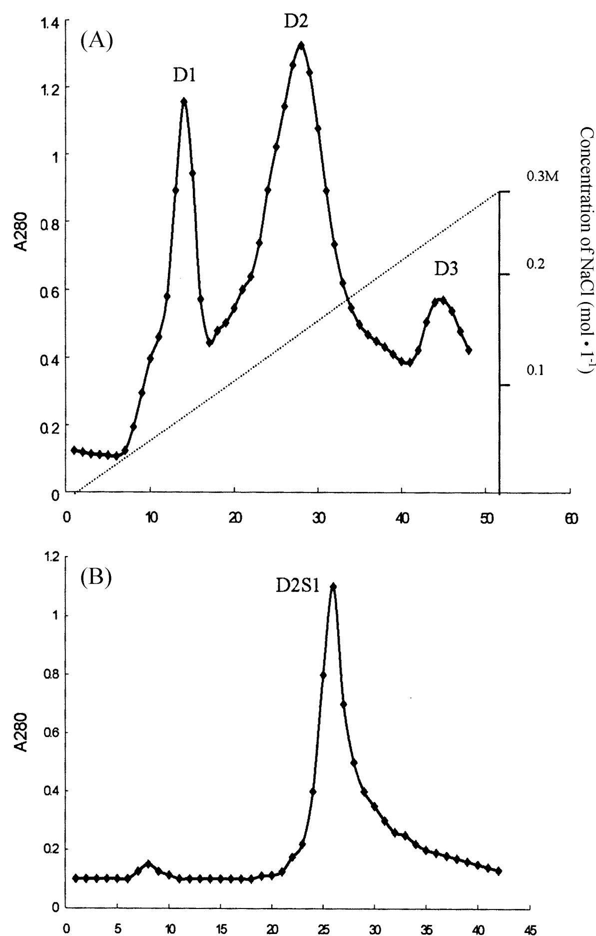

Fig. 1. Purification of AAL by ion-exchang chromatography and gel filtration. (A) Ion-exchange chromatography of the crude protein extract on a DEAE-Sepharose column (1.6 cm × 10 cm), 0-0.3 mol/L NaCl in 10 mmol/L PB buffer (pH 6.0) for gradient elution. (B) Gel filtration of fraction D2 on S-200 column (1.6 cm × 80 cm) eluted with 25 mmol/L PB (pH 7.1) buffer.

Molecular mass and isoelectric point AAL appeared to be homogeneous in SDS-PAGE and in isoelectric focusing (Fig. 2A and Fig. 2B). As shown in Fig. 2A, purified AAL migrated as a single band with a molecular mass of 15.8 kDa. In gel filtration using a Superdex 75 column, AAL showed a molecular weight of 32 kDa in 0.05 M sodium phosphate

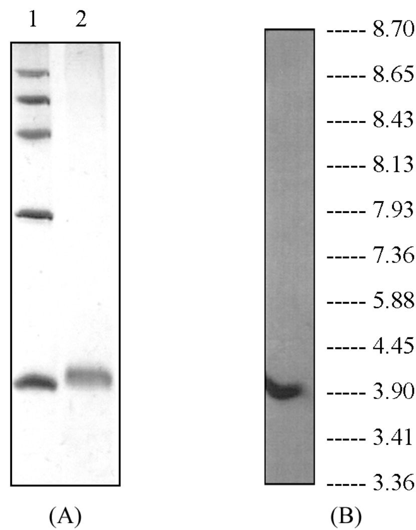

Fig. 2. SDS-PAGE and isoelectric focusing PAGE of *Agrocybe aegerita* lectin (AAL). (A) Lane 1: protein molecular weight markers (94 kDa, 67 kDa, 43 kDa, 30 kDa, 14.4 kDa). Lane 2: *Agrocybe aegerita* lectin (AAL) (B) Isoelectric focusing of AAL on 5% PAGE containing Ampholin 3.0-9.5

buffer (pH 7.0). This result indicated that native AAL was most likely a homodimeric protein. The phenol-sulfuric acid reaction did not give appreciable color characteristic of neutral sugars when up to 2 mg of the lectin was employed, indicating that AAL was devoid of carbohydrate. AAL showed a single band in the pH zone near 3.8 from isoelectric focusing.

Chemical properties The amino acid composition of AAL given in Table 1 showed a high content of glycine, alanine, hydroxylic and acidic amino acids, low content of methionine, phenylalanine, isoleucine and histidine residues, and traces of cysteine. This result was consistent with the low isoelectric point of AAL.

The amino end of native AAL was blocked. By using several methods it was determined that N-terminus of AAL was pyroglutamyl. Being treated by pyroglutamate aminopeptidase, AAL was sequenced from N-terminus as only 8 amino acids QGVNIYNI. No similarity with other published sequences was detected in protein database in respect that the sequence was too short.

Specificity for erythrocytes The hemagglutinating activity of AAL on erythrocytes is shown in Table 2. AAL agglutinated erythrocytes of all human A, B, O types and 12 various animal species. Erythrocytes of human and mammal animal were agglutinated at comparable concentration (equal to titer of $2^{8\text{-}14}$). The hemaggluting titer is $2^{4\text{-}5}$ and $2^{3\text{-}8}$ respectively for avian and aquatic animals. These results suggested that AAL was not specific for any erythrocytes tested; however, the hemagglutinating activity to mammalian red blood cells is much higher than that of avian or aquatic.

Table 1. Amino acid composition of AAL

Amino acid	Content	Residues in one molecule
Asx	8.183	16
Thr	5.637	10
Ser	4.488	8
Glx	6.546	12
Pro	1.674	3
Gly	6.119	11
Ala	6.381	12
Cys	tr.	0
Val	7.496	14
Ile	4.273	8
Leu	5.448	10
Tyr	2.447	5
Phe	3.435	6
Met	0.323	1
Lys	2.201	4
His	0.904	2
Try	ND	ND
Arg	1.780	3

Glx, sum of Glu and Gln; Asx, Asp and Asn; ND, not determined; tr., trace.

Table 2. Erythrocyte agglutination activity of AAL (The initial concentration is $1\ mg \cdot ml^{-1}$.)

Erythrocyte	Titer of agglutination	Erythrocyte	Titer of agglutination
Human (group A)	2^8	Duck	2^5
Human (group B)	2^9	White eel	2^4
Human (group O)	2^8	Turtle	2^4
Rabbit	2^{10}	Yellow eel	2^8
Bovine	2^{14}	Frog	2^5
Sheet	2^{10}	Fish	2^8
Mouse	2^8	Loach	2^5
Chicken	2^3	/	/

2^3=125 μg · ml^{-1} 2^4= 62.5 μg · ml^{-1} 2^5=31.3 μg · ml^{-1} 2^8=3.91 μg · ml^{-1} 2^9=1.95 μg · ml^{-1} 2^{10}=0.97 μg · ml^{-1} 2^{14}=0.06 μg · ml^{-1} as the same as the Table below.

Specificity for sugar-binding Specificity for carbohydrate binding of AAL was examined by a hemagglutination-inhibition assay. The hemagglutinating activities of AAL were not inhibited by the following saccharides up to 200 mM; D-galactose, D-fucose, D-xylose, D-arabinose, D-mannose, D-sorbinose, D-fructose, D-glucose, saccharose, maltose, raffinose, D-galactosamine, dithiothreitol, β-methyl-D-mannoside, β-methyl-D-glucoside, N-acetyl-D-glucosamine. Lactose produced inhibition when it went up to 12.5 mM. Table 3 shows the inhibition of hemagglutinating activity of the lectin by several glycoproteins. Among tested

Table 3. Inhibition of hemagglutination of *Agrocybe lectin* by several glycoconjugates

Glycoconjugates	Concentration ($\mu g \cdot ml^{-1}$) for complete inhibition
Bovine submaxillary mucin (BSM)	7.82
Asialo-BSM	No inhibition at 1000 $\mu g \cdot ml^{-1}$
Glycophorin A	7.82
Asialo-glycophorin A	No inhibition at 1000 $\mu g \cdot ml^{-1}$
κ-Casein	125
Asialo-κ-Casein	No inhibition at 1000 $\mu g \cdot ml^{-1}$
Hog gastric mucine	7.82
Chicken vomucin	No inhibition at 7000 $\mu g \cdot ml^{-1}$
Porcine thyroglobin	No inhibition at 7000 $\mu g \cdot ml^{-1}$

Table 4. The hemagglutination titers of heat-treated lectins

Temperature	Titer of agglutination activity
40ºC	2^{10}
50ºC	2^{10}
60ºC	2^{8}
70ºC	2^{4}
80ºC	0

glycoproteins, BSM, glycophorin A, and hog gastric mucine were strong inhibitiory and to the same extent. κ-Casein produce inhibition at a higher concentration. Asialoglycoproteins (Asialo-BSM, Asialo-glycophorin A, Asialo-κ-Casein) were not inhibitory. Porcin thyroglobulin and ovomucin had no effect.

Stability to temperature, pH and divalent metals The hemagglutinating activity of AAL was stable between 10 and 50ºC. However, the activity decreased quickly above 50ºC, and diminished at 80ºC (Table 4). The activity of AAL was almost completely retained even after being exposured to 0.24 N NaOH or HCl corresponding to pH 12.22 and pH 2.42 (Table 5). The hemagglutinating activity of the lectin was not lowered by demetalization. The salts of divalent metals including $CaCl_2$, $MgCl_2$, or $ZnCl_2$ did not influence the activity of the demetalized lectin either.

Lethaity The LD_{50} of AAL was determined to be 15.85 mg/Kg body weight of mice.

The affection on TMV infection and growth of plant pathology fungi AAL was found to inhibit the infection of TMV on *Nicotiana glutinosa* (Fig. 3). The effect of various concentrations of AAL against TMV infection was shown in Table 6. AAL at concentration of 200 μg/ml produced a 84.3% reduction in lesion number. Only 47.3 and 62.5% inhibition was obtained when the concentration of AAL was 25 or 50 μg/ml, respectively. The 50% inhibition concentration against TMV infection was measured for AAL to be 30-40 μg/ml. To determine the interaction of AAL and TMV, isoelectric focusing was performed as shown in Fig 4. The bands on gel showed that the isoelectric point of AAL and TMV were near 3.8 and 8.7, respectively (lane 1 and lane 2). After 30 min incubation of AAL and TMV (lane 3), the bands of AAL and TMV, were disappeared, and a new band emerged near the location of pH 4.2. The result indicated that AAL had been attached to TMV particles. We suggested that AAL attached to TMV blocking the infecting site was

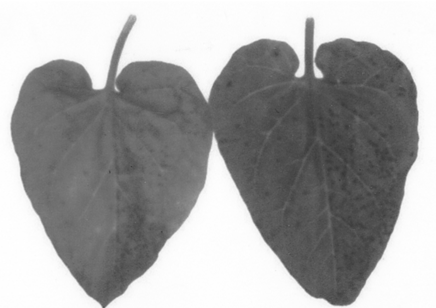

Fig. 3. Inhibition of tobacco mosaic virus (TMV) infection on *Nicotiana glutinosa* leaves by AAL. The left half of the leaves were inoculated with mixture of TMV and AAL. The right half of the leaves were inoculated with mixture of TMV and 0.1 M PB buffer.

Table 5. The hemagglutination titers of acid- and alkali- treated lectins

	HCl (N)				NaOH (N)			
	0.03	0.06	0.12	0.24	0.03	0.06	0.12	0.24
pH	3.02	2.80	2.60	2.42	11.76	11.99	12.01	12.22
Titer	2^{9}	2^{9}	2^{8}	2^{8}	2^{10}	2^{10}	2^{8}	2^{8}

Table 6. Inhibitory effect of AAVP on the infection of TMV on *Nicotiana glutinosa*

Concentration of AAVP ($\mu g \cdot ml^{-1}$)	Inhibition ratio (%)
25	47.3
50	62.5
100	78.7
200	84.3

probable one of the inhibition mechanisms.

However, The growth of fungi mycelia was not inhibited around the AAL paper disk, even at high concentration (10 μg/μl). AAL had no effect on growth of three tested plant pathogen fungi.

Differentiation promotion activity As shown in Fig. 5, mycelia differentiation of two mushrooms *Agrocybe aegerita* and *Auricularia polytricha* were evidently promoted by AAL dripped on the surface of mycelia. As control, mycelia treated by PBS buffer were not changed. The yellow rhizomorph-like structure was formed at the position treated by 0.1 μg/μl AAL, a few fruiting bodies emerged when concentration of AAL went up to 1 μg/μl, more fruiting bodies appeared at 10 μg/μl. The similar phenomenon was observed in *Auricularia polytricha.*

Immunochemical properties Proteins from other five mushrooms were analyzed by Western blot analysis with an antiserum to AAL, and several of them showed positive results. The patterns of SDS-PAGE and Western blot were shown in Fig. 6. The molecular weight of these proteins that reacted with AAL antiserum ranged from 19 to 34 kDa, most of them were near 20 kDa. Two weak bands of molecular weight 23 kDa and 25 kDa were presented in *Pleurotus erygii* and *Ganoderma lucidum*, respectively. A stronger band of 19 kDa was found in *Hericium erinacous*. *Pleurotus erygii* showed two bands, one was 25 kDa and the other was a weak 34 kDa band. The strongest two bands of 24.5 kDa and 34 kDa were observed in *Volvariella volvacea.* Proteins in *Hericium erinacous*, *Lentinula edodes*, and *Volvariella volvacea* show stronger bands with AAL antiserum as compared to proteins in *Pleurotus erygii* and *Ganoderma lucidum.* This result suggested that proteins of *Hericium erinacous*, *Lentinula edodes*, and *Volvariella volvacea* had higher antigenicity than proteins of *Pleurotus erygii* and *Ganoderma lucidum* in the case of the AAL antiserum.

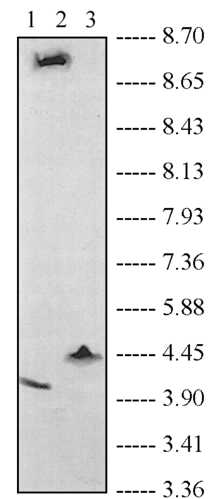

Fig. 4. Detection of interaction between AAL and TMV by isoelectric focusing. Lane1: *Agrocybe aegerita* lectin (AAL). Lane 2: TMV (tobacco mosaic virus) Lane 3: Incubation solution of TMV and AAL.

Discussion

Many mushroom lectins except AAL have been isolated from fresh fruiting bodies or mycelium. Mushroom lectin AAL was

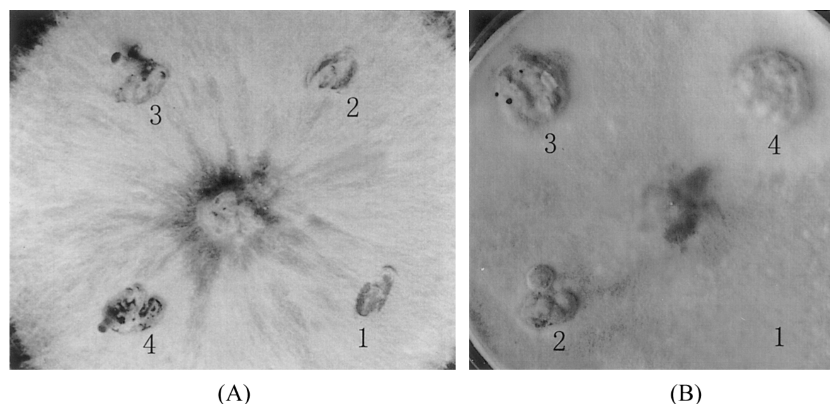

Fig. 5. Differentiation promotion of AAL to mycelia of *Agrocybe agerita* and *Auricularia polytricha*. A, *Agrocybe agerita*; B, *Auricularia polytricha.* 1, Control (PBS); 2, 0.1 μg/μL AAL; 3, 1 μg/μL AAL; 4, 10 μg/μL AAL.

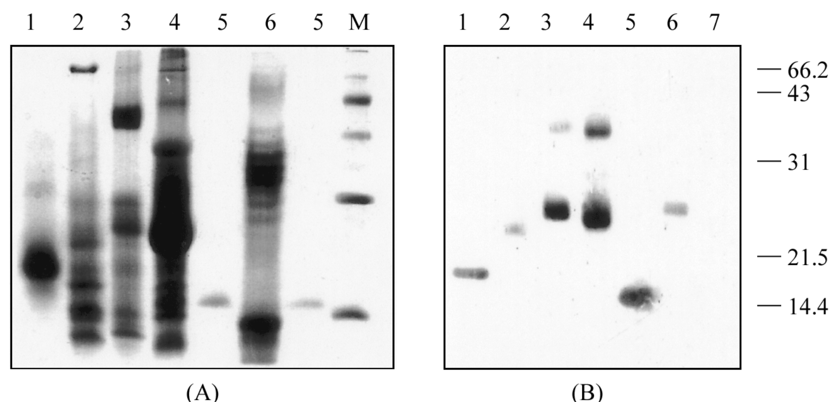

Fig. 6. Western blot analysis of crude protein extraction of five other mushrooms by AAL antiserum. (A) SDS-PAGE of proteins of five other kinds of mushrooms. (B) Western blot analysis. M: protein molecular weight markers (94 kDa, 67 kDa, 43 kDa, 30 kDa, 14.4 kDa). Lane1, *Hericium erinacous*; Lane 2, *Pleurotus erygii*; Lane 3, *Lentinula edodes*; Lane 4, *Volvariella volvacea*; Lane 5, AAL as positive control; Lane 6, *Ganoderma lucidum*; Lane 7, BSA as negative control.

first successfully purified from dry fruiting bodies in this work. Using dry fruiting bodies may be helpful to simplify the purification protocol. This was based on the fact that lectins were more thermostable than many other proteins, which was denatured during the drying process.

Two *Agrocybe aegerita* lectins had been reported by Ticha (1985). The molecular weight of which was 44 kDa, consisting of 2 identical subunits, and its hemagglutinating activity was blocked by Gal. Obviously, AAL-Ticha was distinct from the AAL prepared in this study. Yagi (1997) reported carbohydrate-binding specificity of *A. cylindracea*, which is the synonym with *A. aegerita*. By comparing AAL with AAL-Yagi, we found that they are probably the same protein. The AAL-Yagi made up of two identical subunits, each 15 kDa in size, was very similar to AAL reported in this paper in molecular structure. The amino acid composition was almost the same and the behavior on ion-exchange chromatography was similar. The character of N-terminal block was the same. In hemagglutinin inhibition tests, the concentrations of the glycoproteins for complete inhibition were the same within experimental error. In this paper, we reported other biochemical and novel physiological characteristics, which was helpful to study the role of AAL played *in vivo*.

Like most mushroom lectins, AAL was rich in hydroxylic amino acid and poor in methionine, histidine, and phenylalamine. But its low pI (3.8) was special in mushroom lectins, while we know little about other characteristics corresponding to its low pI.

The hemagglutinating activity of AAL was preserved after incubation at 50ºC. At 60 and 70ºC, very little was remained and at 80ºC or above it was completely abolished. Thus, AAL was less thermostable than several mushroom lectins such as TML (*Tricholoma mongolicu* lectin) (Wang *et al.*, 1995), VVL (*Volvariella volvacea* lectin) (Lin *et al.*, 1984), and HEL (*Hericium erinaceum* lectin) (Kawagish *et al.*, 1994). However, the hemagglutinating activity of AAL was almost not changed after exposure to high concentration NaOH and HCl. Resistance of AAL to extreme pH treatment was a very interesting result and deserves further studies. AAL was toxic to mice and its LD_{50} is 15.85 mg/kg. The N-terminal block, stability to extreme pH, and toxicity to mice (LD_{50}) were very similar with *Vovariella volvacea* lectin, which was fully active at pH 2 or 11 and has an LD_{50} of 17.5 mg/kg (Lin *et al.*, 1984). Nevertheless, no homology was found in their N-terminal amino acid sequence.

TMV infection inhibition of AAL is an astonishing observation. A fruiting body protein (FBP) as TMV infection inhibitor was isolated from *Lentinus edodes* by Kobayashi in 1987 (Kobayshi *et al.*, 1987); FBP did not attach to TMV and characteristics of which was not described. With the exception of type II RIPs (ribosome inactive proteins) no other lectins had been reported to inhibit viral infection, replication, or systemic spread (Peumans *et al.*, 1995). The behavior of AAL: inhibition of TMV infection, attachment to TMV, and having no effect on growth of fungi, were very similar to that of PAP (Pokeweed antiviral protein, a type I RIPs). PAP inhibited TMV infection by interacting with TMV (Kumon *et al.*, 1990) and inactivating ribosome of tobacco (Taylor *et al.*, 1994), but had no effect on growth of bacteria and fungi (Chen *et al.*, 1991). While the molecular structure of AAL was neither consistant with type I or type II RIPs. Thus, AAL may be a novel type of antiviral protein. The detail of the interaction mode of AAL and TMV is an interesting subject on account of the coat protein of TMV is a pure protein without sugar linked. To elucidate antiviral function of AAL, more investigations are expected.

Plant lectin was accumulated as part of nitrogen reserve and used as passivity-defense proteins because of the abundance of lectins in storage organs, thus it showed antibacterial, antifungal and deleterious effects on predatory invertebrates and high animals (Peumans *et al.*, 1995).

High production of lectins in many mushrooms such as AAL, TML, VVL, and TEL, indicated that lectins exists as a kind of storage protein in mushroom. The toxicity of VVL and AAL to mice and antiviral activity of AAL described in this paper give the evidence that lectins may be involved in mushroom's defense. Thus, mushroom lectins may play similar defense roles in mushrooms like plant lectins play in plant.

The differentiation promotion activity of AAL was another exciting finding. Many observations have been suggested that the appearance of lectins was closely correlated with the fruiting body formation. It has been reported that the fruiting body but not the vegetative mycelium of *Flammulina velutipes* (Yatohgo *et al*., 1998) contained a lectin protein, and the developmental appearance of lectin was observed in *Coprinus cinereus* and *Pleurotus cornucopiae* (Kaneko *et al.*, 1993). The synthesis of AAL was not limited to fruiting bodies, but the concentration in mycelia was much lower than in fruiting bodies (data not shown). In present work, the formation of fruiting bodies was dose dependently accelerated by AAL dripped on the mycelium surface, provide the powerful direct evidence to support the viewpoint that mushroom lectins play important roles in fruiting body formation. From this study we supposed that AAL was an actor more than a product in the process of mycelia differentiation.

It must be pointed out that AAL was not the only determinative factor in formation of fruiting bodies. One third of mycelium differentiation assays failed because of the change of temperature and humidity. These results suggested that mushroom lectin was one of the main factors in fruiting body formation, while it was also obviously affected by other conditions such as temperature or humidity. The precise mechanism of AAL in differentiation promotion to mycelia needs to be confirmed by further investigation, but we have found other indications that it agglutinated spores and mycelia of *Agrocybe aegerita*.

Results of immunoblotting showed that several proteins in five mushrooms gave cross reactions with the AAL antiserum. The mushrooms tested in Western blot belong to six different families; *Hericium ramosum* and *Ganoderma lucidum* even belonging to other orders. These results suggest that proteins with similar immunological properties to AAL exist in most mushrooms, made up a large protein family and probably play some unknown roles.

The specific activities of AAL determined in this experiment and the fact that proteins of other mushrooms cross-reacted with AAL antiserum indicate that a large protein family with similar immunological properties to AAL may play various physiological roles *in vivo*.

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