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Purification and characterization of an *N*-acetyl-D-galactosamine-specific lectin from the edible mushroom *Schizophyllum commune*

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Abstract

An *N*-acetyl-D-galactosamine (GalNAc)-specific lectin was purified from the edible mushroom, *Schizophyllum commune*, using affinity chromatography on a porcine stomach mucin (PSM)-Sepharose 4B column. Under reducing and non-reducing conditions, SDS-polyacrylamide gel electrophoresis gave a major band of 31.5 kDa. The *Schizophyllum commune* lectin (SCL) showed high affinity toward rat erythrocytes and the sugar inhibition assay exhibited its sugar specificity highly toward lactose and *N*-acetyl-D-galactosamine. It was stable at 55 °C for 30 min and at pH 3–10 for 18-h test. The lectin was shown to be a glycoprotein with cytotoxic activity against human epidermoid carcinoma cells. The N-terminus of SCL was blocked but amino acid sequences of internal tryptic peptides showed moderately sequence similarities with some other fungal and plant lectins. Crystals of SCL were obtained by the sitting drop vapour-diffusion method using polyethylene glycol 8000 as the precipitant, and gave an X-ray diffraction pattern to approximately 3.8 Å resolution.

Keywords: Lectin; Edible mushroom; *N*-acetyl-D-galactosamine-specific lectin; Lectin crystal; *Schizophyllum commune*; X-ray diffraction

1. Introduction

Lectins are a heterogeneous group of proteins or glycoproteins of non-immune origin that specifically and reversibly bind to carbohydrates of glycoconjugates [1,2]. These proteins are ubiquitous in nature, and occur in animals, plants, bacteria, viruses, and fungi [3,4]. Most lectins play a crucial role in diverse biological processes, particularly in host defense mechanisms, inflammation, and metastasis [5]. Owing to their binding specificities, lectins are employed in numerous biochemical and clinical research areas [6–8]. *N*-acetyl-D-galactosamine (GalNAc)-specific lectins are of a great interest since they have been reported as a detecting agent for tumor-associate antigens (Thomson–Friedenreich or T-antigens) of malignant cells [9,10].

Lectins with high affinity to GalNAc have been isolated and characterized from both vertebrates and invertebrates. For the purpose of the anticancer drug production, work on GalNAc lectins has been rapidly increasing. A novel mouse macrophage (M∅) C-type lectin (MGL) which has Gal/GalNAc-specificity has been well characterized [11,12]. The cDNA cloning of GalNAc-specific lectin from starfish, *Asterina pectinifera* was also attempted. Most recently, X-ray crystallographic analysis of a sea cucumber, *Cucumaria echinata* lectin (CEL), which recognizes GalNAc, was revealed [13].

In addition, several fungal lectins, which possess immunomodulatory, mitogenic, and antitumor activities, have been isolated, for example from *Agaricus bisporus*, *Volvariella volvacea*, *Grifola frondasa*, and *Tricholoma mongolicum* [14–17]. The discovery of lectin in a mushroom from tropical areas with cytotoxic activity could possibly be highly interesting.

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Although some of GalNAc-specific lectins have been intensively studied, only a few reports on crystallization of the GalNAc-specific lectin from edible mushrooms have been published.

In the present study, a GalNAc-specific lectin from the edible mushroom, *Schizophyllum commune* collected from a local market in Thailand was purified and characterized. The cytotoxic property of its lectin was elucidated. Additionally, single crystals of the *Schizophyllum commune* lectin (SCL) were prepared for the first time, and proved suitable for X-ray diffraction studies.

2. Materials and methods

2.1. Purification of the SCL

Fresh fruiting bodies of the edible mushroom *Schizophyllum commune* were obtained from a local market in Nakhon Pathom Province, Thailand. The mushroom specimens were dried at 40 °C for 2 days. The dried specimens were ground into powder using a blender and stored in sealed plastic bag at temperature below 25 °C until use. The lectin was extracted by homogenizing the mushroom powder with 10 times (w/v) of 10 mM phosphate buffer saline (PBS), pH 7.2, containing 1 mM benzamidine, 0.1% (v/v) 2-mercaptoethanol, and 1.5% (w/v) polyvinylpyrrolidone (PVPP) and stirring overnight at 4 °C. The homogenate was filtered through cheesecloth, and the filtrate was centrifuged at 14000×*g* for 30 min at 4 °C. Then solid ammonium sulfate was added to the supernatant to obtain 30% saturation. After stirring overnight at 4 °C, the supernatant was collected by centrifugation at 100000×*g* for 30 min at 4 °C. The supernatant was loaded on to a porcine stomach mucin (PSM)-Sepharose 4B column which was equilibrated with 10 mM Tris–HCl, pH 8 containing 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 0.02% (w/v) NaN_3. Unbound proteins were eluted and the lectin was desorbed with 20 mM 1, 3-diaminopropane (DAP). All fractions were then neutralized with 1 M Tris–HCl, pH 7. The adsorbed fractions were assayed for hemagglutinating activity before repeating the loading on to the column in order to increase their purity. Then the final fractions were represented as purified lectin.

2.2. SDS-PAGE

SDS-PAGE was performed on 17.5% (w/v) polyacrylamide gel containing 0.1% (w/v) of SDS according to the method of Laemmli [18]. After electrophoresis, the gel was stained with Coomassie brilliant blue R-250. The molecular weight of the purified lectin was determined by comparing its electrophoresis mobility with the standard molecular weight marker proteins, LMW-SDS marker (Amersham-Pharmacia Biotech, Uppsala, Sweden). In order to determine carbohydrate of the lectin, periodic acid Schiff's (PAS) staining was investigated according to the method described by Segrest and Jackson [19].

2.3. Protein content estimation

Protein concentration was determined by the method of Bradford [20].

2.4. Enzymatic deglycosylation

In order to determine the *N*-glycosidic linkage of the SCL, the deglycosylation was performed using the Glycoprofile II, enzymatic in-solution *N*-deglycosylation kit (Sigma, St. Louis, USA). Then molecular mass of the lectin subunits after deglycosylation was detected by SDS-PAGE.

2.5. Amino acid sequence analysis

After SDS-PAGE, the lectin band was immobilized on a polyvinylidene difluoride (PVDF) membrane by electroblotting, which was performed at 14 V for 30 min with a semi-dry transfer cell for N-terminal sequencing by Edman degradation using an Applied Biosystems model 471A Protein Sequenator. In-gel digestion was carried out using an adaptation of the method of Rosenfeld et al. [21]. Gel slices from SDS-PAGE were washed (2×30 min) with 50% acetonitrile, 0.2 M ammonium bicarbonate pH 8.9 and then freeze-dried. The slices were re-swollen in 50 μl of RHB (0.2 M ammonium tricarbonate pH 7.8, 0.02% tween 20)/2 M urea containing 0.5 μg trypsin and incubated at 37 °C overnight. Excess RHB/2 M urea was then removed to an Eppendorf tube and peptides were extracted from the gel slices with 2 lots of 60% acetonitrile and 0.1% TFA. The latter and the excess RHB/2M urea were pooled, concentrated by centrifugal evaporation, and applied to a reverse phase HPLC column to separate the peptides. Suitable peptides were then subjected to sequencing by Edman degradation using a model 471A Protein Sequenator (Applied Biosystems, Warrington, UK).

2.6. Heat stability and pH stability tests

The heat stability and pH stability of the selected lectin was examined according to the modified method of Freire et al. [22] and Kobayashi et al. [23]. Heat stability was determined by heating aliquots of the lectin (1 mg/ml lectin in PBS) for 30 min at various temperatures of 40, 50, 55, 65, 80, and 100 °C. Then the heated aliquots were cooled rapidly on ice, centrifuged to remove any precipitate, and assayed for hemagglutination in comparison with a control sample of lectin. Results were expressed by calculating the percentage of hemagglutination shown by the heated aliquots (titration value) compared with the control sample representing 100%.

For the pH stability test, the lectin was measured by incubating the lectin samples (1 mg/ml) in the following buffers varying from pH 3–10 for 18 h at 4 °C. Different buffers were used according to pH range as follows; 50 mM glycine–HCl buffer (pH 2.0–3.0), 50 mM sodium acetate buffer (pH 4.0–5.5), 50 mM Tris–HCl buffer (pH 8.0–8.5), and 50 mM glycine–NaOH buffer (pH 9.0–11.0). The residual hemagglutinating activity was assayed after dialysis the lectin samples against PBS for 18 h at 4 °C. Three replicates were done for each test. Results were calculated by expressing the titration values of the lectin as percentages of the titration value of the control.

2.7. Hemagglutinating activity and sugar inhibition assays

A serial two-fold dilution of the mushroom lectin solution (50 μl) in microtiter U-plates was mixed with the same volume of a 2% suspension of rabbit erythrocytes in PBS, pH 7.2 and incubated at 4 °C. The results were read after about 1 h when the negative control had fully sedimented. The hemagglutination titer, defined as the reciprocal of the highest dilution exhibiting hemagglutination, was reckoned as one hemagglutination unit.

Sugar inhibition assay was investigated for studying sugar specificity of the mushroom lectin. The sugar-inhibition of lectin-induced hemagglutination using various sugars was performed in a manner analogous to the hemagglutination test. Serial two-fold dilutions of test sugar samples were prepared in PBS, pH

Table 1
Hemagglutinating activities of SCL chromatographic fractions from 15 g of dried fruiting body powder against rabbit erythrocytes

Step	Total protein (mg)	Specific hemagglutinating activity (unit/mg)	Total hemagglutinating activity (unit)	Recovery of hemagglutinating activity (%)	Fold of purification
Crude extraction	7548	86.5	652,800	100	–
30% NH_2SO_4 precipitation	4371	87.9	384,000	58.8	1.0
PSM-Sepharose 4B affinity column	32.4	2496.1	80,640	12.4	28.9

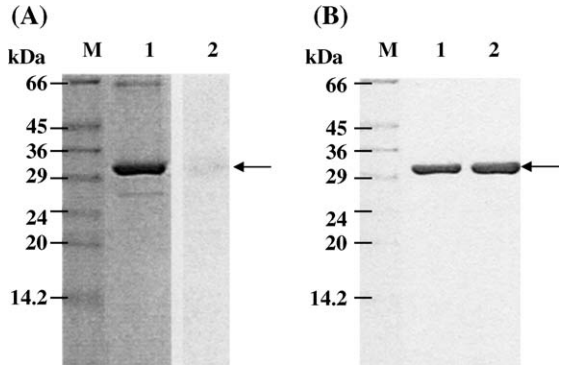

Fig. 1. SDS-PAGE of SCL. (A) Lanes: M, molecular weight marker proteins (LMW-SDS marker, Amersham-Pharmacia Biotech); 1 and 2 purified lectin that was stained by Coomassie brilliant blue and the periodic acid Schiff's reagent, respectively. (B) Lanes: M, molecular weight marker proteins; 1, 2, purified lectin after deglycosylation.

7.4. Each dilution was mixed with an equal volume (25 μl) of a solution of the lectin with 16 hemagglutination (HA) units. The mixture was allowed to stand for 30 min at 4 °C before mixing with 50 μl of 2% rat (or rabbit) erythrocyte suspension. The minimum concentration of the test sugar in the final reaction mixture, which completely inhibited 16 hemagglutination units of the lectin, was recorded.

2.8. Antimicrobial activity test

Test organisms used for the antibacterial assay were *Bacillus cereus* ATCC 6633, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 10145, and *Escherichia coli* ATCC 25922. For inoculum preparation, the test culture strains were transferred from nutrient agar slants into test tubes containing nutrient broth, and grown overnight by shaking at 37 °C. Then the overnight culture was diluted with sterile PBS, pH 7.4, to yield a 0.5 McFarland suspension. The prepared inoculum was then aseptically inoculated on Mueller–Hinton agar plate by three-dimension swab technique. The filter paper discs were each impregnated with 50 μl of the purified lectins and placed on the inoculated agar then incubated at 37 °C for 24 h. The filter paper disc containing the reference antibiotics (ampicillin) (10 mg/ml) was used as a positive control [24].

For the antifungal activity assay, *Saccharomyces cerevisiae* ATCC 26108, *Candida albican* ATCC 10231, *Aspergillus niger* ATCC 6275, and *Penicillium digitatum* ATCC 201167, were grown in 2% (w/v) malt extract broth (MEB) for 48 h at 30 °C, then streaked on 2% (w/v) MEA for the agar diffusion experiments. The concentration of the fungal suspension tested was adjusted to 10^7 cells or spores/ml. The lectin extract was applied as described above. One hundred units of nystatin were used as positive control. In this test, inhibitory zones were detected after incubation for 48 h at 30 °C [24]. All tests were also performed in triplicates.

2.9. Cytotoxicity test against cancer cell lines

Human epidermoid carcinoma (KB) and human cervical carcinoma (HeLa) cell lines were investigated. These cells were trypsinized before seeding at a density of 2×10^4 cells/100 μl in 96 well plates for 24 h, at 37 °C, in an atmosphere of 5% CO_2. Then serial concentrations of the lectin extract (100 μl/well) were added before further incubation for 72 h (the same as previous condition). At the end of the incubation, the medium was removed and the plate was washed with PBS. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) solution (100 μl/well) was then added following by further incubation for 4 h. Absorbance at 550 nm was measured using microtiter reader after incubation with dimethyl sulfoxide (100 μl/well) for 30 min. Reagents and controls were included with the absence of cells or the crude extract, respectively. The 50% inhibitory concentration (IC_{50}) values which less than 30 μg/ml were designated as cytotoxicity.

2.10. Lectin crystallization

Schizophyllum commune lectin crystals were grown using the sitting drop technique using an Innovadyne '96 plus 8' crystallization robot in a series of crystallization trials using JBScreen HTS I (PEG based) and JBScreen HTS II (ammonium sulfate, MPD, alcohol and salt based) reagent kits (Jena Bioscience, CA). A total of 96 different crystallization drops were set up for each screening test using 'Intelliplates' (Hampton Research, CA, USA) to establish the likely conditions for crystal growth. Each sitting drop was a mixture of 0.5 μl of lectin (10 mg/ml) and 0.5 μl of the screening reagent, and 160 μl of the same screening reagent was used in the well as a reservoir.

X-ray diffraction experiments were carried out at 100 K using the synchrotron radiation source at the Council of the Central Laboratory of the Research Councils (CCLRC) Daresbury Laboratory, UK on SRS Station 14.1 equipped with an ADSC Q4 charge-coupled device (CCD) system.

3. Results

In the first stage of the mushroom lectin extraction, polyvinylpyrrolidone (PVPP) was used to overcome the problem of polyphenol, which was previously observed to interfere with protein absorbance at 280 nm. The PSM-Sepharose 4B column was loaded with 150 ml of the crude extract. After the activity was completely adsorbed, the column was washed with Tris–HCl, pH 8. Then the bound lectin was eluted with 20 mM DAP run through the column. The eluates were collected in 6 ml fractions and immediately neutralized with 1 ml of 1 M Tris–HCl buffer, pH 7. To remove all impurities in the bound fractions, they were pooled together and rerun with the same column and then the purified lectin was collected. A summary of the extraction and purification yields of the lectin is given in Table 1. The specific activity of the crude extract was obtained at 86 units/mg, but after purification by a double step of the affinity chromatography, the specific activity of the lectin was increased up to 2496 units/mg. From 15 g of dried mushroom powder, 32.4 mg of lectin were obtained. By this purification protocol, a 28-fold purification could be achieved.

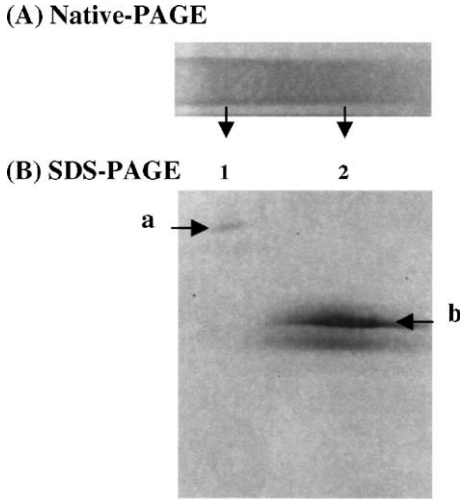

Fig. 2. Two-dimension gel electrophoresis of SCL. (A) Native gel-PAGE of SCL eluted from the porcine stomach mucin-Sepharose 4B column. (B) SDS-PAGE of two fractions from the native gel-PAGE; Lanes: 1, homodimer (band a); 2, purified lectin (band b).

Both in the presence or absence of 2-mercaptoethanol, SDS-PAGE showed the same results (Fig. 1A), with a strong sharp band of the lectin corresponding to an apparent molecular weight of 31.5 kDa, and a faint band of 29 kDa.

For native-PAGE study, native gel electrophoresis was carried out on 29:1 acrylamide gel and run at 25 mA. Bands cut from the native gel were soaked and run on SDS-PAGE for two-dimensional analysis. From the first run of the native gel, a sharp band plus a more diffuse band were obtained (Fig. 2). After the bands of interest were cut into a gel segment and run on SDS-PAGE, the diffuse band gave a strong band of SCL, which was a glycoprotein, and another weaker band of co-purified lectin that might correspond to unglycosylated SCL. The sharp band in another lane gave a band with higher molecular weight, possibly corresponding to the homodimer. When deglycosylation of the purified lectin was carried out, only single band of 31.5 kDa was still detected by SDS-PAGE (Fig. 1B). This result implied that the weaker band was not unglycosylated form of the lectin. Furthermore, the periodic acid Schiff's (PAS) assay showed that the strong band was a glycosylated protein, but probably was not highly glycosylated. Thus, only a faint pink band was noticed.

From the preliminary hemagglutination assay, the crude extract of *Schizophyllum commune* was shown to agglutinate both rat and rabbit erythrocytes at 128 and 106 titers with rat and rabbit erythrocytes, respectively. In this study, the rabbit erythrocytes were selected for specific activity test and sugar inhibition assay in order to facilitate comparison between the lectin activity of this SCL and other mushroom lectins that have been previously studied even though its titer was lower than rat erythrocytes. In the binding-specificity assay (Table 2), D-galactose, GalNAc, and lactose showed inhibitory activity against the hemagglutination of rabbit erythrocytes by SCL at 100, 0.78, and 0.78 mM, respectively. Some di- and oligosaccharides such as 2′-fucosyllactose (Fucα1→2Galβ1→4Glc), 3-fucosyllactose (Galβ1→4(Fucα1→3)Glc), and lactitol (Galβ1→4Glcitol), were also inhibitory at 100, 100, and 50 mM, respectively. Other sugars tested including L-rhamnose, L-arabinose, D-xylose, D-glucose, D-mannose, raffinose, Me-α-Gal, Me-β-Gal, *p*-nitrophenyl-*N*-acetyl-α-D-galactosaminide (*p*-nitrophenyl-α-GalNAc), *p*-nitrophenyl-*N*-acetyl-β-D-galactosaminide (*p*-nitrophenyl-β-GalNAc), *p*-nitrophenyl-α-D-galactopyranoside (*p*-nitrophenyl-α-Gal), *p*-nitrophenyl-β-D-galactopyranoside (*p*-nitrophenyl-β-Gal), and galactosamine showed no inhibitory effect on the lectin even at high concentration up to 200 mM.

Peptide A: I Q G C V G G R D E
Peptide B: G T P I I G W D Y X E X
Peptide C: T F L A D I K P G X

Fig. 3. Three peptide sequences of the purified SCL obtained from a tryptic digestion.

After the SCL band (approximately 50 μg of the purified lectin) from the tricine SDS-PAGE gel was blotted onto a PVDF membrane and applied to the automated protein sequencer, no amino acid peak was obtained. It indicated that this SCL had a blocked N-terminus. However, a tryptic digest of the SCL was also attempted in order to determine the partial amino acid sequence. Protein fragments fractionated by reverse phase HPLC were selected for sequencing. The SCL partial sequences (Fig. 3) were submitted to the NCBI BLAST database and sequence similarities with lectins from *Marasmius oreades*, *Polyporus squamosus*, and *Arabidopsis thaliana* were observed at 66, 53, and 46%, respectively (Table 3). However, no significant homology with those of the mouse macrophage

Table 2
Hemagglutination inhibition assay on SCL (16 HA units) by various sugars

Test sugars	Concentration of sugar in mmol/l											
	200	100	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.17	PBS
D-Glucose	−	−	−	−	−	−	−	−	−	−	−	−
D-Galactose	+	+	−	−	−	−	−	−	−	−	−	−
N-Acetyl-D-galactosamine	+	+	+	+	+	+	+	+	+	−	−	−
Mannose	−	−	−	−	−	−	−	−	−	−	−	−
Xylose	−	−	−	−	−	−	−	−	−	−	−	−
Fucose	−	−	−	−	−	−	−	−	−	−	−	−
Raffinose	−	−	−	−	−	−	−	−	−	−	−	−
Arabinose	−	−	−	−	−	−	−	−	−	−	−	−
Rhamnose	−	−	−	−	−	−	−	−	−	−	−	−
Lactose	+	+	+	+	+	+	+	+	+	−	−	−
Me-α-Gal	−	−	−	−	−	−	−	−	−	−	−	−
Me-β-Gal	−	−	−	−	−	−	−	−	−	−	−	−
p-Nitrophenyl-*N*-acetyl-α-D-galactosaminide	−	−	−	−	−	−	−	−	−	−	−	−
p-Nitrophenyl-*N*-acetyl-β-D-galactosaminide	−	−	−	−	−	−	−	−	−	−	−	−
p-Nitrophenyl-α-D-galactopyranoside	−	−	−	−	−	−	−	−	−	−	−	−
p-Nitrophenyl-β-D-galactopyranoside	−	−	−	−	−	−	−	−	−	−	−	−
Galactosamine	−	−	−	−	−	−	−	−	−	−	−	−
Galβ1→4Glcitol (Lactitol)	+	+	+	−	−	−	−	−	−	−	−	−
Fucα1→2Galβ1→4Glc (2′-Fucosyllactose)	+	+	−	−	−	−	−	−	−	−	−	−
Galβ1→4(Fucα1→3) Glc (3-Fucosyllactose)	+	+	−	−	−	−	−	−	−	−	−	−

+, hemagglutination inhibition; −, no hemagglutination inhibition; PBS, phosphate buffer saline.

Table 3
Comparison of the partial amino acid sequences of SCL with known lectins

	Position of the first amino acid sequence	Sequence	Identity (%)	Positives (%)
SCL	1	GTPIIGWDY	66	88
MOA	29	GTPIVGWQF		
SCL	16	GGRDEGTPIIGWD	53	61
PSL	27	GSGQNGTPVIAWD		
SCL	5	DIKPGXIQGCVGGRD	46	73
ATPP	814	DVEPGIVEGSVGTED		

MOA: *Marasmius oreades* agglutinin-gi|18476512|gb|AAL47680.1|; PSL: *Polyporus squamosus* lectin-gi|34915974|dbj|BAC87876.1|; ATPP: *Arabidopsis thaliana* putative protein-gi|7263614|emb|CAB81580.1|. The number after each protein corresponds to a number in protein database.

(M∅) C-type lectin (MGL), starfish lectin, and sea cucumber lectin were found, although some of them had similar carbohydrate specificity [11–13].

Although crude extract of SCL showed some antifungal activities against *Aspergillus niger* ATCC 6275, no activity was observed in the purified lectin even at the highest concentration of 1.0 mg/ml. However, the cytotoxic activity of SCL was tested against human epidermoid and cervical carcinoma cell lines. It was shown that SCL has high selective cytotoxic activity against human epidermoid carcinoma cells with an IC_{50} value of 20 μg/ml but not against human cervical carcinoma cells.

The results from heat stability and pH stability studies demonstrated that SCL was not heat-stable but stable to pH changes over a wide range. The activity started to decrease at 55 °C and is completely lost at 65 °C after 30 min. The lectin activity was not markedly affected by pH, with the maximum activity being retained at pH 5.0 to 9.0.

For crystallization, JBScreen I gave many more hits of SCL crystals, compared with JBScreen II. Crystals of different morphologies grew under different conditions and appeared within one to two weeks. The typical plate-like growth of the crystals were obtained with the reservoir buffers containing 18% PEG 8000, 0.1 M Na HEPES pH 7.5, 0.2 M Ca acetate or 15% PEG 8000, 0.1 M Na MES pH 6.5, 0.2 M Na acetate. The crystals grew up to 0.1 mm in the largest dimension. X-ray diffraction data extended to a maximum resolution of 3.8 Å (Fig. 4). Optimization experiments for crystals diffracting to higher resolution are underway.

4. Discussion

According to our preliminary purification of the *Schizophyllum commune* lectin, it has been found that the PSM-Sepharose 4B contained terminally linked GlcNAcα1→4Gal and also GalNAc residues [25,26], was the most effective for the purification of the lectin compared with other four different affinity columns of desialylated hog gastric mucin-Sepharose 4B, PSM-Sepharose 4B, desialylated PSM-Sepharose 4B, and *N*-acetyl-D-galactosamine (GalNAc). High purity and quantity of the lectin was obtained. The PSM-Sepharose 4B column proved to be a suitable affinity chromatography medium for the purification of the lectin from the tropical mushroom, *Schizophyllum commune*, and it has been successfully used for isolation of the fungal lectin from *Rhizopus stolonifer* [27].

Although *Schizophyllum commune* mushrooms are closely related to *Pleurotus ostreatus*, desialylated hog gastric mucin affinity chromatography could not be used for its purification (data not shown). This finding suggests that a carbohydrate-specificity difference of the lectins between these mushroom families is definitely significant for the efficiency of the purification for the purpose of, for example, crystallization.

This lectin could agglutinate both rabbit and rat erythrocytes and showed a little higher activity with rat erythrocytes. SCL had a weak specificity towards galactose, but much stronger specificity towards GalNAc and lactose. In this respect, it was similar to the galactose-specific fungal lectins of *Pleurotus cornucopiae* and *Pleurotus ostreatus* [28–33], GalNAc-specific lectins of *Grifola frondosa* [34], *Lactarious delicious* [35], and *Agrocybe cylindracea* [36], and the lactose specific lectin of *Hygrophorus hypothejus* [37]. However, only cytotoxic activity of the lectin from *Grifola frondosa* against HeLa S_3 cells was reported.

According to this study, the purified SCL was characterized as a GalNAc-specific glycosylated lectin, which contains a subunit with molecular weight of 31.5 kDa, and forms a homodimer, which could be detected in 2D gel electrophoresis. The SCL was not *N*-linked glycoprotein, but it was a dimeric lectin. The SCL is similar to other mushroom lectins, particularly *Pleurotus ostreatus* [28]. The molecular weight of the SCL was also close to the fucose-specific lectin extracted from *Aleuria aurantia*, which is composed of two identical subunits of 36 kDa [38–40] whereas others are composed of multiple subunits, for example, *Agaricus campestris* lectin and *Hericium erinaceum*, which are tetrameric [41,42]. Staining of SDS-PAGE gel with periodic acid Schiff's reagent yielded positive results with a faint band indicating that the portion of carbohydrate in the molecule was much less than the portion of protein.

Among saccharides tested, the sugars having galactosyl or *N*-acetylgalactosaminyl groups inhibited hemagglutination of the SCL, and some derivatives of *N*-acetylgalactosamines were more active than that of galactoses. These results indicate that

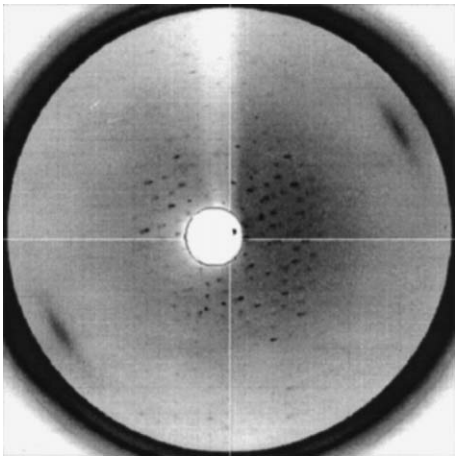

Fig. 4. Diffraction pattern from a crystal of SCL.

SCL recognizes a galactosyl residue, and the specificity was increased by substitution at C-2 position of the galactosyl residue with acetyl-amino group. Even if the inhibitory effect of 2′-fucosyllactose and 3-fucosyllactose was observed, their specificity was not higher than GalNAc. Thus, the SCL probably did not bind to the fucosyl group but to the galactosyl group in both 2′-fucosyllactose and 3-fucosyllactose. Interestingly, the *p*-nitrophenyl groups attached to C1 of galactose ring did not increase the inhibitory potency. These were different from most plant lectins that the hydrophobic interactions established between amino acid residues in the vicinity of the carbohydrate-binding site and the *p*-nitrophenyl group, enhance the affinity of the complex [43].

Although the N-terminus of SCL was blocked as is the case for the other mushrooms e.g. *Agrocybe cylindracea* [36], *Grifola frondasa* [34], and *Mycoleptodonoides aitchisonii* [44], some internal amino acid sequences of tryptic peptides were determined. The BLAST analysis demonstrated some sequence similarity with other fungal and plant lectins. Even the percent similarity of these lectins was not very high, this information is still essential not only for further three dimensional structure and specific recognition mechanism studies of the mushroom lectin but also for the evolutionary study of the lectin family.

Crystals of the edible mushroom lectin, SCL, were obtained and some X-ray diffraction patterns were recorded. The sitting drop technique was performed with a crystallization robot. This instrument was a valuable tool for the crystallization of an unknown protein. Only 0.5 μl of purified lectin was required for each crystallization trial, so that many conditions could be screened. The SCL crystals were ~0.1 mm in their longest dimension. X-ray diffraction results from this preliminary test also show clearly that these crystals contain protein. However, to improve the quality of diffraction data, further crystallization trials are needed.

Recombinant mistletoe lectin, ML, was reported to belong to a group of galactoside-specific/lactose-binding lectins, and defined as a Type II ribosome-inactivating protein, RIP [45]. SCL might possibly yield to the same process, to help with further studies. It may also be developed as a potent anticancer agent.

In conclusion, this is the first report of purification and structural characterization of a lectin from *Schizophyllum commune*, an edible mushroom widely grown in Thailand. Single crystals of the SCL were successfully prepared and proved to be suitable for X-ray diffraction studies. The SCL showed its cytotoxic activity against human cancer cells so the lectin deserves to be a potential agent for cancer treatment. This information will be very useful for structure based drug design in the future.

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