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Extraction, purification and physicochemical properties of a novel lectin from *Laetiporus sulphureus* mushroom

Yufeng Wang[a,*], Benyue Wu[a], Jiangjuan Shao[b], Jinxia Jia[a], Youqiu Tian[a], Xu Shu[a], Xiaojie Ren[a], Yue Guan[a]

[a] College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China
[b] College of Pharmacy, Nanjing University of Traditional Chinese Medicine, Nanjing 210046, China

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ABSTRACT

Technological conditions for extracting *Laetiporus sulphureus* lectin (LSL) were optimized through an orthogonal experiment. LSL, with a relative agglutinating activity of 259.90 ± 7.47 UH/mg protein, was successfully obtained, and its structure was characterized. Results indicated that the novel 52.0 kDa LSL was rich in aspartic acid, glutamic acid, leucine, and threonine, and its secondary structure mainly presented alpha-helix, beta-sheet, beta-turn, and random-coil conformation. Moreover, its relative agglutinating activity was inhibited by alpha-lactose and beta-lactose. Meanwhile, D-maltose, D-glucose, D-fructose, D-mannose, and D-xylose did not exert this effect. The obtained LSL showed resistance to acid and alkali and demonstrated moderate thermostability, and its relative agglutinating activity was reduced by Fe^{3+} and Al^{3+} ions. Meanwhile, Ca^{2+}, Mg^{2+}, Zn^{2+}, and Mn^{2+} ions had no inhibitory effects.

1. Introduction

Laetiporus sulphureus is an edible and medical fungus. Its shelf-shaped fruiting body is pink-orange and has a fleshy bright yellow outline (Weber, Mucci, & Davoli, 2004). This remarkable pigmentation accounts for its specific epithet and trivial name, sulfur shelf. This cosmopolitan polypore fungus causes brown rots in the stems of mature and overmatures trees in forests and urban areas. *L. sulphureus* can be cultivated at large scale and in laboratories. Furthermore, it can be cultivated as a fruiting body in a solid-state surface culture and as a mycelium in a submerged culture (Davoli, Mucci, Schenetti, & Weber, 2005). This fungus contains various metabolites, such as polysaccharides, *N*-methylated tyramine derivatives, and lanostane triterpenoids (Turkoglu, Duru, Mercan, Kivrak, & Gezer, 2007; Weber et al., 2004), and produces significant compounds, such as antioxidant, anti-inflammatory, antiviral, antimicrobial, and cytostatic agents and HIV-1 reverse transcriptase inhibitors (Steinberg, Poran, & Shapira, 1999; Xue et al., 2001). Thus, it has been extensively investigated and used over the past years.

Lectins constitute a class of proteins with at least one noncatalytic domain that binds specifically and reversibly to different types of glycoproteins, monosaccharides, or oligosaccharides. Lectins are capable of specific recognition and reversible binding with carbohydrates (Peumans & Van Damme, 1995). These proteins are ubiquitously distributed in nature and abundant in the Plantae kingdom. Many lectins from plants have remarkable biological functions, such as antifungal and antiviral effects, nitrogen-fixing bacteria recognition, cell agglutination, and mitogenic stimulation (Charungchitrak, Petsom, Sangvanich, & Karnchanatat, 2011; Sharon & Lis, 2004; Zhang, Shi, Ilic, Jun Xue, & Kakuda, 2008). Current research revealed that lectins not only exhibit defense and antitumor functions but also promote and regulate mitosis, cell response and intercellular substance-mediated response (Kang, Lee, Chung, Choi, & Kim, 1982; Ngai & Ng, 2004; She, Ng, & Liu, 1998; Turkoglu et al., 2007; Yu, Fernig, & Rhodes, 2000). Lectin from *L. sulphureus* is composed of subunits associated by non-covalent bonds and exhibits hemagglutination and hemolytic activities. Owing to these properties, LSL solutions are frequently prepared for crystallization experiments. However, the structure of LSL has not been fully explored. Mancheño et al. isolated a hemolytic lectin from *L. sulphureus* by performing affinity chromatography on Sepharose (Mancheño, Tateno, Goldstein, & Hermoso, 2004). Native and recombinant forms of lectin showed hemagglutination and hemolytic activity, which were inhibited by *N*-acetyllactosamine (Chang, Liu, & Chen, 2011). Meanwhile, Li et al. focused on the practical application of LSL and used it as a potent affinity tag. Highly specific antibodies against LSL were already developed (Li et al., 2016). However, research on LSL is currently limited and nonsystemic.

In this study, LSL was extracted and purified, and its molecular

* Corresponding author.
E-mail address: joywangyu@sina.com (Y. Wang).

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weight and amino acid composition were investigated through Fourier transform infrared spectroscopy (FT-IR) and circular dichroism spectroscopy (CD). Additionally, its molecular morphology was characterized by atomic force microscopy (AFM). The stability of LSL was evaluated by observing the influence of sugars, pH, temperature, and positive ions on its relative agglutinating activity. Our research would provide a promising and functional component for food and medicine.

2. Materials and methods

2.1. Materials

L. sulphureus thalli were obtained from Chibei Forest Treasure-purchasing Station (Changbai Mountain Protection and Development Zone, CGMCC5.617, China). Unless stated otherwise, all the chemicals and reagents used in our experiments were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Deionized water was used throughout the experiments and purified by a Mill-Q water purification system from Millipore (Bedford, MA, USA).

2.2. Animals

Experiments were carried out in male New Zealand rabbits weighing 1000.00 ± 25.00 g (Nanjing University of Traditional Chinese Medicine, China). The rabbits were kept in a temperature-controlled room (24 ± 1 °C), given free access to standard-compliant diets (whole value grain feed stuff) before the experiments, and acclimatized for at least one week. All the experiments were performed in accordance with the internationally accepted guidelines on laboratory animal use.

2.3. Preparation of rabbit blood cell suspension

For blood sampling, the rabbits were first anesthetized, and their blood was immediately collected from their central auricular arteries with syringes prefilled with citrate (3.8%; 1:9, v/v) (Zhao, Wang, & Ng, 2009). The obtained blood samples were centrifuged at 500 × g for 5 min. The pellets were washed with normal saline (0.9%; 1:5, v/v) five times and adjusted to 2% (v/v) in citrate for experimental use.

2.4. Preparation of crude LSL

L. sulphureus thalli (100.00 g) were cleaned, lyophilized (a Model 2K-XL Lyophilizer, Virtis Corporation, America), ground with a Model ZN-100 Grinder (Zhongnan Pharmaceutical Machinery Factory, Shanghai, China), and filtered through a 20-mesh sieve. Thallus powder (85.00 g) was obtained and mixed with PBS (0.01 mol/L) and sodium chloride. The resulting solutions were successively subjected to the following treatments: designed concentrations of sodium chloride, 0–1.00 mol/L, solvent (PBS) to raw material extraction ratio, 10.0–50.0 mL/g; extraction temperature, 4–45 °C; extraction time, 5–25 h; and pH, 6.0–8.0. Each aqueous extract was centrifuged (Model 5804R, Eppendorf Corporation, Germany) at 4680 × g for 10 min until a supernatant solution was obtained, which was subsequently stored at 4 °C for the subsequent experiments.

2.5. Orthogonal experimental design

The optimum extracting conditions of crude LSL were determined through orthogonal tests. A single factor experiment was conducted, and agglutination activity and protein content were used as indices. Four main factors (sodium chloride concentration, PBS to raw material ratio, extraction temperature, and extraction time) were selected, and their proper ranges were determined. A nine-run orthogonal experimental design with four factors and three levels was implemented, and its relative agglutination activity was used as the index (Table 1).

Table 1
Orthogonal experiment and variance analysis.

a. Orthogonal experiment

Run	A (concentration, mol/L)	B (ratio, mL/g)	C (temperature, °C)	D (time, h)	Relative agglutinating activity (UH/mg protein)
1	1(0)	1(20:1)	1(15)	1(10)	5.57
2	1(0)	2(30:1)	2(25)	2(15)	3.83
3	1(0)	3(40:1)	3(35)	3(20)	5.14
4	2(0.25)	1(20:1)	2(25)	3(20)	7.61
5	2(0.25)	2(30:1)	3(35)	1(10)	6.09
6	2(0.25)	3(40:1)	1(15)	2(15)	4.95
7	3(0.50)	1(20:1)	3(35)	2(15)	7.21
8	3(0.50)	2(30:1)	1(15)	3(20)	7.12
9	3(0.50)	3(40:1)	2(25)	1(10)	6.73
k_1	4.84	6.80	5.88	6.13	
k_2	6.24	5.68	6.06	5.33	
k_3	7.02	5.61	6.15	6.62	
R	2.18	1.19	0.27	1.29	

b. Variance analysis

Source	Sum of squares	Degree of freedom	F-ratio	F marginal value	Significance
A	7.278	2	66.771	19.00	*
B	2.657	2	24.376	19.00	*
C	0.109	2	1.000	19.00	
D	2.554	2	23.431	19.00	*
Error	0.11	2			

*Significant difference ($p < .05$).

2.6. Purification of LSL

Crude LSL were first salted out with ammonium sulfate (Guan et al., 2015). The saturation level of ammonium sulfate in the crude LSL (200.0 mL) was subsequently adjusted stepwise to 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%. The solution was left for 5 h at 4 °C. The sediment was isolated through centrifugation at 4680 × g for 15 min at 4 °C. The solution was dissolved in PBS (0.01 mol/L, pH 7.5) for further dialysis, then desalted against distilled water, and finally lyophilized. The optimum saturation of ammonium sulfate was identified on the basis of the relative agglutinating activity of lectin as an indicator.

LSL (0.05 g) was dissolved in PBS (5.0 mL) and applied to a column of diethyl-aminoethanol (DEAE)-cellulose-52 (Φ 26.0 × 300.0 mm). The resulting solution was subjected to stepwise elution with 0, 0.15, 0.30, 0.50, and 1.00 mol/L sodium chloride solutions (300.0 mL, added with PBS) at a flow rate of 0.48 BV/h. The eluate (1.0 mL/tube) was collected automatically. Absorbance was measured at 280 nm in a Model 752 UV spectrophotometer (Shanghai Jinghua Instrument Co, Ltd., Shanghai, China). The main fractions were collected, concentrated, dialyzed, and lyophilized sequentially, and their relative agglutinating activities were measured.

Lectin with the highest relative agglutinating activity was selected and purified through high performance gel filtration chromatography (HPGFC). A Water 1525 HPLC pump (Waters Corporation, USA) equipped with Waters Ultrahydrogel linear (Φ7.8 mm × 300.0 mm) and a model 2414 refractive index detector (RID). The partially purified fraction (0.01 g) was dissolved in PBS (1.0 mL), injected into the detector, and eluted with PBS at a flow rate of 0.9 mL/min. The eluent was detected at 280 nm. The main fraction was collected, concentrated, dialyzed, and lyophilized.