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A novel core 1 O-linked glycan-specific binding lectin from the fruiting body of *Hericium erinaceus*

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ABSTRACT

Mucin-type *O*-glycans are involved in biological functions on the cell surface as well as the glycoproteins and can also be used as specific carbohydrate biomarkers of many diseases. In this study, I purified a novel core 1 *O*-linked glycan specific lectin, *Hericium erinaceus* lecin (HeL), from the fruiting body of the mushroom *Hericium erinaceus*, which is known as the natural source for a sialic acid-binding lectin. Upon optimization of the purification conditions, a sequence of ion exchange, affinity, ion exchange, and size-exclusion chromatography resulted in the highest yield and best quality of lectin without protease activity. The resulting purified HeL is an apparent hexameric protein with a subunit molecular weight of 15 kDa, and a pI of 4.3. In hemagglutination inhibition assay, the purified lectin was only inhibited by glycoproteins containing mucin-type *O*-glycans and reacted weakly with Galβ(1,3)GalNAc. Glycan array analyses showed that HeL specifically interacts with core 1 *O*-linked glycans as well as extended *O*-glycan structures containing sialylation or fucosylation. The glycan binding specificity of HeL is comparable to that of peanut agglutinin for detection of a broader range of extended core 1 *O*-glycan structures. Taken together, these results provide an efficient and optimized procedure for the purification of HeL from the fruiting body of the mushroom *Hericium erinaceus*. Moreover, HeL represents a powerful tool for analyzing core 1 and extended core 1 *O*- glycan structures in diagnosis assays.

1. Introduction

Lectins are specific carbohydrate-binding proteins (GBPs) of non-immune origin. These proteins agglutinate animal red blood cells and precipitate glycoconjugates. Moreover, GBPs exhibit enormous diversity, complexity, and flexibility in their binding of glycans and glycoconjugates, which are found in dense and complex layers decorating the cell surfaces of organisms and are also involved in cellular and molecular interactions. These agglutination proteins play roles in several important biological processes, including the mediation of protein quality control via glycan-specific binding activities, host-pathogen interactions, cell–cell communication, inflammation, immune responses, tissue metastasis, and development [1].

Fungi, including mushrooms, microfungi, and yeasts, are attractive resources in the search for a novel lectin that recognizes the unique carbohydrate structures in glycan and glycoconjugates for therapeutic and biotechnological applications [2,3]. Interestingly, more than 80% of reported fungal lectins have been identified from mushroom species [2]. Moreover, mushroom lectins exhibited binding specificity for complex glycan structures (glycoconjugates) consisting of monosaccharides with various linkages and side chain modifications [4,5].

Hericium erinaceus (mistakenly called *Hericium erinaceum*) is an edible and medicinal mushroom found in East Asia (China, Korea, and Japan). An *N*-glycolylneuraminic acid (Neu5Gc)-binding lectin has been identified in the fruiting body of the mushroom [5]. In the report of its discovery [6], *H. erinaceum* lectin (HEL) is described as a 54 kDa heterotetrameric protein consisting of two different

Abbreviations: BSM, bovine submaxillary mucin; CV, column volume; Fuc, fucose; Gal, galactose; GalNAc, N-acetylgalactosamine; GBPs, carbohydrate-binding proteins; GlcNAc, N-acetylglucosamine; HEL, *Hericium erinaceum* lectin; HEA, *Hericium erinaceum* agglutinin; HeL, *Hericium erinaceus* lectin; MALDI-TOF MS, matrix assisted laser desorption ionization-time of flight mass spectrometry; MOA, *Marasminus oreades* agglutinin; MS, mass spectrometry; Neu5Ac, N-acetylneuraminic acid; Neu5Gc, N-glycolylneuraminic acid; PMSF, phenylmethylsulfonyl fluoride; PNA, peanut agglutinin; PSM, porcine submaxillary mucin; TF, Thomsen-Friedenreich.

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subunits, which are 15 kDa and 16 kDa in size. HEL exhibits glycan-binding activity that is more effective at agglutination against porcine erythrocytes than against other animal red blood cells. On the other hand, in a subsequent report [7], *H. erinaceum* agglutinin (HEA) is described as a 51 kDa intact monomeric protein consisting of a single subunit. The N-terminal sequence of HEA was AFGQLS-FANLAAADF. The hemagglutination activity of HEA toward rabbit erythrocytes was strongly inhibited solely by inulin among the 21 carbohydrates tested. These differences between HEL and HEA show that the fruiting body of *H. erinaceus* likely harbors several isolectins with unique carbohydrate binding activities. In addition, these variations in specific agglutination activities might contribute to the regional species differentiation of the mushroom *H. erinaceus* in Japan and China. However, the amino acid sequence identities of these lectins are still unknown.

In this study, I isolated and characterized a novel lectin with specific binding activity for core 1 *O*-linked glycan structures from a molecularly identified *H. erinaceus* strain. Here, I present an optimized purification procedure for core 1 *O*-linked glycan binding lectin from the fruiting body of the mushroom and compare the purified lectin's physicochemical properties with those of other agglutination proteins previously reported in this mushroom.

2. Materials and methods

2.1. Materials

The fruiting bodies of *H. erinaceus* were obtained from a local mushroom farm in Korea. After purchase, the fruiting bodies were frozen and stored at −80 °C. Fetuin-agarose was purchased from Sigma-Aldrich. DEAE-Sepharose Fast Flow, HiTrap™ Q FF and Superose 12 10/300GL were purchased from GE Healthcare. Animal blood products were purchased from Innovative Research (Novi, MI). All chemicals used in this study were of analytical reagent grade.

2.2. Molecular identification of mushroom samples

Hericium erinaceus mushrooms obtained from a local mushroom farm were molecularly identified by analyzing the rDNA locus. To identify *H. erinaceus* strain, PCR was carried out with NL-1 and NL-4 primer pair and the ITS-1 and ITS-4 primer pair for amplification of 26S rDNA D1/D2 and 630 bp segment of the ITS1-5.8S rDNA-ITS4 locus from its genomic DNA, respectively. The details of the protocol and results are described in supplementary methods and data (supplementary data 1, Fig. S1).

2.3. Preparation of lysates of the mushroom fruiting body

The frozen fruiting bodies were ground into a fine powder under liquid nitrogen in a mortar. The powdered frozen fruiting bodies were suspended in 10 mM Tris-HCl buffer (pH 8.0) containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and cOmplete™ EDTA-free protease inhibitor cocktail (Roche, Switzerland) and extracted overnight. The homogenate was centrifuged at 10,000*g* for 30 min, and the resulting supernatant was centrifuged at 20,000 × g for 60 min. The clarified crude lysate was used as the starting material for lectin purification.

2.4. Affinity chromatography purification

For affinity chromatography, crude protein solution was loaded onto an affinity column (2.6 × 15 cm) packed with fetuin-agarose (Sigma-Aldrich, F3256) that had been equilibrated with 10 mM Tris-HCl buffer (pH 8.0) containing 1 mM PMSF and a protease inhibitor cocktail. After washing the column with five times its volume of the same buffer, the lectin proteins were eluted with three times the bed volume of 10 mM Tris-HCl buffer, followed by a step gradient of 20 mM, 40 mM, 100 mM, and 200 mM galactose. Fractions (8 mL) were collected at a flow rate of 1 mL/min. The fractions with hemagglutination activity were pooled, concentrated by ultrafiltration, and then loaded onto the next column.

2.5. Optimization of a lectin purification protocol from the fruiting body lysate of H. erinaceus

The crude protein solution was applied to a DEAE-sepharose column (2.6 × 20 cm) equilibrated with 10 mM Tris-HCl buffer (pH 8.0) containing 1 mM PMSF and a protease inhibitor cocktail. After unbound proteins were washed using five column volumes of buffer, the protein fractions were eluted with three bed volumes of the buffer, followed by a linear gradient of 0.0-1.0 M NaCl or a step gradient of 0.25 M, 0.5 M, 1.0 M, and 2.5 M NaCl. Fractions (8 mL) were collected at a flow rate of 1 mL/min. The fractions with agglutination activity were pooled, concentrated by ultrafiltration, and loaded onto the next column.

These active pools were applied to a fetuin-agarose column that had been equilibrated with 10 mM Tris-HCl buffer (pH 8.0). After washing the column with five times its volume of the same buffer, the lectin proteins were eluted with three times the bed volume of 10 mM Tris-HCl buffer containing 250 mM galactose at a flow rate of 1 mL/min. The fractions that exhibited hemagglutination activity were pooled and applied to the next column.

Then the active protein pool was loaded onto a HiTrap™ Q FF column (0.7 × 2.5 cm) (GE Healthcare) equilibrated with 10 mM Tris-HCl buffer (pH 8.0) and eluted with thirty bed volumes of the buffer, followed by a linear gradient of 0.0–1.0 NaCl. Fractions (0.5 mL) were collected at a flow rate of 0.5 mL/min.

Finally, the concentrated active protein pool (1.0 mL) was applied to a Superose 12 10/300 GL (GE Healthcare) column equilibrated with phosphate-buffered saline (PBS) (pH 7.4). The proteins were eluted with the same buffer and fractions (0.5 mL) were collected at a flow rate of 0.5 mL/min. The active fractions were analyzed using SDS-PAGE or tricine-PAGE, pooled and concentrated by ultrafiltration.

2.6. Hemagglutination activity and inhibition assays

The hemagglutination activity of lectin was determined using a two fold serial dilution procedure with intact erythrocytes, as follows: 50 μL lectin-containing solution was mixed with an equal volume of 4% (v/v) formaldehyde-treated porcine red blood cells suspended in PBS in individual wells of 96-well microtiter plate with V-shape wells (Greiner Bio one, Monroe, NC), and a two fold serial dilution in PBS was performed down the plate. The mixture was left to stand for 1 h at room temperature.

For the hemagglutination inhibition assay, crude lysate or purified lectin protein was incubated with sugars or glycoproteins at room temperature for 1 h and then assayed for residual hemagglutination activity. The hemagglutination titer of an individual sugar or glycoprotein was defined as the reciprocal of the highest dilution exhibiting hemagglutination.

2.7. N-terminus sequence analysis and peptide mass fingerprinting

The purified proteins were transferred onto an Immobilon™ polyvinylidene fluoride (PVDF) transfer membrane (Millipore, Bedford, MA). The N-terminal sequences of the purified lectins were analyzed via the Edman degradation method by the eMass analytical laboratory (Seoul, Korea).