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ScienceDirect
Phytomedicine 16 (2009) 352–360
Phytomedicine
www.elsevier.de/phymed

A novel sialic acid-specific lectin from *Phaseolus coccineus* seeds with potent antineoplastic and antifungal activities

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Abstract

A novel lectin (PCL) with specificity towards sialic acid was purified from *Phaseolus coccineus* L. (*P. multiflorus willd*) seeds using ion exchange chromatography on CM and DEAE-Sepharose, and gel filtration on Sephacryl S-200 column. PCL was a homodimer consisting of 29,831.265 Da subunits as determined by gel filtration and MS. Also, PCL was a non-metaloprotein and its N-terminal 23-amino acid sequence, ATETSFSFQRLNLANLVLNKESS, was determined. Subsequently, MTT method, cell morphological analysis and LDH activity-based cytotoxicity assays demonstrated that PCL was highly cytotoxic to L929 cells and induced apoptosis in a dose-dependent manner. Using caspase inhibitors, a typical caspase-dependent pathway was confirmed. PCL also showed remarkable antifungal activity towards some plant pathogenic fungi. Furthermore, when sialic acid-specific activity was fully inhibited, cytotoxicity and antifungal activity were abruptly decreased, respectively, suggesting a significant correlation between sialic acid-specific site and its bi-functional bioactivities.

Keywords: *Phaseolus coccineus* lectin; Sialic acid specificity; Apoptosis; Caspase-dependent; Antifungal activity

Introduction

Lectins are carbohydrate-binding proteins that occur throughout the whole biosphere. Also, they have been found to possess many significant biological activities such as antineoplastic, immunomodulatory and antifungal activities (Sharon and Lis, 1989; Engering et al., 2002). Apoptosis is a genetically regulated self-destructive cellular death process that is important in numerous cancer diseases (Liu et al., 2008b). Owing to the apoptotic mechanism, numerous lectins have been utilized in the alternative tumor therapy for several years (Stauder and Kreuser, 2002). Until now, most sialic acid-specific lectins have been purified from invertebrates, and only a few plant-derived lectins that specifically bind to sialic acids have been studied as potential antifungal agents (Takashi and Ajit, 2002).

In the present study, we reported for the first time that a novel sialic acid-specific lectin from the *Phaseolus coccineus* seeds was isolated and characterized. Interestingly, besides hemagglutinating activity, we discovered that PCL showed remarkable antineoplastic and antifungal activities. These results would provide new evidence for understanding more significant biological implications of PCL in further investigations.

Materials and methods

Chemicals and reagents

L929 cells were provided by Medical Sciences Center of West China, Sichuan University. RPMI 1640 and

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doi:10.1016/j.phymed.2008.07.003

fetal bovine serum (FBS) was purchased from Gibco-BRL (New York, USA). DEAE-Sepharose, Sephacryl S-200 and standard molecular weight markers were procured from Pharmacia (Uppsala, Sweden). Saccharides and glycoproteins, acridine orange (AO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), aprotinin, L-glutamine and leupeptin were products of Sigma Chemicals (St. Louis, MO, USA). z-DEVD-fmk, z-IETD-fmk, z-LEHD-fmk, z-AEVD-fmk and z-VAD-fmk were purchased from Sigma Chemicals (St. Louis, MO, USA). Standardized kinetic determination kit and LDH kit was purchased from Zhongsheng (Beijing, China).

Plant material

P. coccineus (*P. multiflorus willd*) seeds were obtained from Dali, Yun'nan, China.

Purification of lectin

Approximately 50 g of the seeds were powdered. The powder was soaked overnight at 4 °C with 0.02 M NaAc–HAc buffer (pH 4.4). The mixture was then centrifuged at 20,000*g* for 15 min. The resulting supernatant obtained was loaded on a column of CM-Sepharose previously equilibrated and eluted with 0.02 M NaAc–HAc buffer (pH 4.4). After washing of unadsorbed materials, the column was eluted sequentially with 0.1, 0.15, 0.3, and 0.6 M NaCl in the same buffer to collect adsorbed fractions. Fractions which contained hemagglutinating activity were dialyzed against 0.02 M Tris–HCl buffer (pH 8.0); then the samples were applied on a DEAE-Sepharose column previously equilibrated and eluted with 0.02 M Tris–HCl buffer (pH 8.0). After washing of unadsorbed material devoid of hemagglutinating activity, a peak with hemagglutinating activity was eluted with 0.15, 0.3 and 0.6 M NaCl in the same buffer. Then the active fractions were pooled and loaded on a column of Sephacyl S-200 in 0.02 M PBS (pH 7.0).

Determination of molecular mass

The purified PCL was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for subunit molecular mass determination as described by Laemmli and Favre (1973). The native molecular mass was estimated by gel filtration chromatography on the Sephacryl S-200 column, according to the method of Andrews (1964).

Mass spectrometry analysis

Matrix-assisted laser desorption/ionization time-of-flight (MALDI–TOF) mass spectrum was obtained using a Voyager-RP mass spectrometer (PerSeptive Bio-systems) according to the method of Woo et al. (2001).

Determination of N-terminal sequence

The N-terminal sequence of the lectin polypeptides was carried out by using a Hewlett-Packard HP G1000A Edman degradation unit and an HP 1000 HPLC System (Lam et al., 1998).

Assay for hemagglutinating activity

A serial two-fold dilution of the lectin solution in microtiter U-plates (50 µl) was mixed with 50 µl of a 2% suspension of rabbit red blood cells in phosphate-buffered saline (pH 7.2) at 20 °C. The results were read after about 1 h when the blank had fully sedimented. The hemagglutination titer, defined as the reciprocal of the highest dilution exhibiting hemagglutination, was reckoned as one hemagglutination unit. Specific activity is the number of hemagglutination units per mg protein (Wang et al., 2000).

Carbohydrate-binding specificity

For determining the sugar-binding specificity of this lectin, sugars including arabinose, D-mannose, D-fructose, D-arabinose, L-rhamnose, L-xylose, L-sorbose, D-galactose, sialic acid, D-glucose, sucrose, lactose, raffinose, maltose, D-galactosamine, L-fucose, N-GlcNAc, N-GalNAc, glycoproteins were tested for their ability to inhibit lectin-induced hemagglutination. Lectin concentration, just upstream the end point titre of hemagglutination, was chosen. Thirty microlitres of test lectin and equal volume of 100 mM sugar or 2 mg/ml of glycoprotein was incubated at 37 °C for 1 h.

Metal ion requirement

Demetallization of purified lectin was performed by the method of Paulova et al. (1971). The activity in normal and demetallized samples was compared by the hemagglutination assay.

Thermal and pH stability

These tests were conducted as previously described for the mushroom (*Tricholoma mongolicum*) lectin (Wang et al., 1995, 1996).

Cell culture

PCL was dissolved in dimethyl sulfoxide (DMSO) below 0.1% which had no detectable effect on cell