Note

Isolation and Characterization of N-Acetylgalactosamine-specific Lectin from Galactia tashiroi Seeds

Nobuhiko Fukuda, Atsushi Yoshimaru, Toshiro Hidaka, Hideaki Ohta,* Kyosuke Yamamoto,** and Harugoro Yomo†

Laboratory of Food Science and Nutrition, Faculty of Agriculture, Miyazaki University, Miyazaki 889-21, Japan
*Chugoku National Agricultural Experiment Station, Ministry of Agriculture, Forestry and Fisheries, Fukuyama, Hiroshima 721, Japan
**Department of Internal Medicine, Saga Medical College, Saga 849, Japan

Received June 28, 1993

An N-acetylgalactosamine-specific lectin was isolated from Galactia tashiroi seeds by affinity chromatography on Galactose-Sepharose 6B. It was a glycoprotein with molecular weight of 90,000-95,000 and composed of four identical subunits of molecular weight of 24,000. The purified lectin was specific for N-acetyl-D-galactosamine, while it was non-specific for human type ABO erythrocytes.

Lectins are carbohydrate-binding proteins that agglutinate erythrocytes and other normal or transformed cells, but they have a wide diversity in their specificity for carbohydrate. They are useful in the isolation and analysis of glycoproteins, in cell separation, and in assessing the structural properties of the cell surface.1-3 Most lectins are found in either plant or animal tissues, and are especially common in the seeds of leguminous plants.2,3

During a survey of various leguminous seeds native to Okinawa, we found that extracts of the seeds of Galactia tashiroi (GT) show a marked hemagglutinating activity for human O type erythrocytes.4 Although extracts from the seeds of Galactia filiformis and G. striata have been shown to agglutinate human type ABO erythrocytes nonspecifically,5 nothing has been reported about the purification of lectins from Galactia species. In this study, we describe the purification and partial characterization of a N-acetyl-D-galactosamine-specific lectin from the seeds of GT.

Mature seeds of GT found in Okinawa were collected and stored at -25°C until use. Sepharose 6B, Sephadex G-100, and Sephadex G-150 were obtained from Pharmacia Chemicals. Biogel P-6DG was obtained from Bio-Rad. A standard protein (cross-linked cytochrome c) for SDS-polyacrylamide electrophoresis was obtained from Oriental Yeast Co., Ltd. The sugars and other chemicals used were from Wako Chemicals and were of analytical grade.

In measuring hemagglutinating activity and inhibition of hemagglutination by sugars, we used freshly prepared 4% human type O erythrocyte suspensions in phosphate-buffered saline (PBS, 0.15M NaCl containing 0.01M sodium phosphate buffer, pH 7.4), using methods described elsewhere.4,6 Activity was assessed visually and expressed as a titr, which is the reciprocal of the highest dilution of lectin showing detectable agglutination. Protein and carbohydrate concentrations were measured by the absorbance at 280 nm and the phenol-sulfuric acid method7 using bovine serum albumin and glucose as standards, respectively. Purification was done at 5°C.

Finely ground seeds were defatted with ethyl ether, followed by mixing with PBS for 2 h at 4°C with constant stirring. After the extract had been clarified by centrifugation, the supernatant was put on a column (2.6 × 15 cm) of galactose-Sepharose 6B prepared by the method of Iglesias et al.8 and equilibrated with PBS. The non-adsorbent fractions were eluted with PBS until absorbance at 280 nm was less than 0.02, and the adsorbed proteins were then eluted with 0.1M galactose in PBS. The fractions containing hemagglutinating activity were combined and dialyzed against PBS extensively, and then passed through a Biogel P-6DG column (2.5 × 50 cm) to remove any remaining galactose.

The purified lectin thus obtained was shown to be homogenous on disc-gel electrophoresis9 at both pH 4.0 and pH 9.4, and on ultracentrifugation (Fig.). The sedimentation coefficient of the lectin, calculated for water at 20°C and zero time concentration, is 7.3. Assuming a e of 0.75, a molecular weight of 90,000 was obtained by the sedimentation equilibrium method. Attempts to measure the molecular weight of GT lectin on Sephadex G-100 or Sephadex G-150 were unsuccessful, because the purified lectin that was put on the column emerged as a very broad and faint peak, in close agreement with the hemagglutinating activities at a position far after the void volume. When the lectin was electrophoresed on an SDS-polyacrylamide gel10 after heat denaturation in the presence or the absence of 2-mercaptoethyl, it migrated as a single band to a position corresponding to a molecular weight of 24,000 and 95,000, respectively. These results indicate that the GT lectin has a molecular weight of 90,000-95,000 and is a tetramer composed of four apparently identical subunits of 24,000. The results also suggest that each subunit is linked by disulfide bonds. Total sugar content of the lectin was 3.2%, indicating that the lectin is a glycoprotein. The molecular properties of the GT lectin are similar to those of known N-acetyl-D-galactosamine-specific lectins isolated from the seeds of such legumes as Dolichos biflorus,11 Glycine max,12 and Crotalaria striata13; these lectins are all glycoproteins with molecular weights of 110,000-140,000 and a tetrameric composition of four apparently identical subunits.

Table shows the inhibitory effects of various sugars on hemagglutination by purified GT lectin. Hemagglutinating activity of the lectin was specifically inhibited by N-acetyl-D-galactosamine; the inhibitory activity of this aminosugar was 32 times more potent than galactose or α-D-mannopyranoside. There was no difference between the inhibitory activities of the α and β anomers of C-1-substituted galactosides, such as methyl and α-nitrophenyl derivatives. The hydroxymethyl group at C-6 of the pyranose ring appeared to be important for inhibition, as shown by the fact that 1-arabinose, which has the same configuration of hydroxyl groups as in galactose, is not inhibitory. N-acetyl-D-glucosamine

† Formerly Professor of the University of the Ryukyus, Faculty of Agriculture, Nishihara, Okinawa 982-01. Present address: Asahiyaoka-cho, Nishihara-shi, Hyogo 659, Japan.
showed a partial inhibition and was as potent as galactose. Considered together with the potent inhibitory activity of N-acetyl-
D-galactosamine, the substitution of an acetamido group for the
hydroxyl group at the C-2 of the pyranose ring appeared to be
important for inhibition. A faint, but definite, inhibition of
hemagglutination by glucose and maltose was observed, perhaps
related to the interaction of the GT lectin with Sephadex G-100
or Sephadex G-150 described above.

The agglutination specificity of the lectin was analyzed by use
of 4% erythrocyte suspensions prepared from various animal
species. The lectin had an equal hemagglutinating activity against
human type ABO erythrocytes at a minimum concentration of
2 μg per ml, which was consistent with the observations of Boyd
et al.51 on crude seed-extract. With rabbit and rat erythrocytes,
the concentration of lectin required to cause agglutination was
64-fold higher that was required with human type O erythrocytes.

The stability of GT lectin in PBS (512 titer, mg/ml) was in-
vestigated under different conditions of pH14 and tempera-
ture.151 The lectin was found to be stable from pH 4.0–10.0 at

5°C for 1 day and to withstand heating up to 70°C for 60 min.
Isoelectric focusing of GT lectin in a linear sucrose gradient in
the range pH 3–10 yielded a single symmetric protein and activity
peak with a maximum at pH 8.0.

Since the GT lectin is the first to be isolated from a Galactia
species, we could make no comparison with other lectins from
this species. GT lectin differs with respect to its specificity for
carbohydrate and human ABO group erythrocytes from the
N-acetyl-D-galactosamine-specific lectins mentioned above; those
two lectins except for Glycine max are all group A-specific, and
do not interact with N-acetyl-D-glucosamine, Sephadex G-100,
or Sephadex G-150.

In summary, we isolated a new lectin specific for N-acetyl-D-
galactosamine from the seeds of Galactia tashiroi. Further studies
of its physicochemical properties are underway.

References
1. I. J. Goldstein, R. C. Hughes, M. Monsigny, T. Osawa, and N.
5. W. C. Boyd, E. Waszczenko-Zachczenko, and S. M. Goldwasser,
Transfusion, 1, 374–382 (1961).
7. M. Dubois, K. A. Giles, J. K. Hamilton, P. A. Rebers, and P. Smith,
(1982).
12. H. Lis, B. A. Sela, L. Sachs, and N. Sharon, Biochim. Biophys. Acta,
(1982).
15. L. Bhattacharyya, A. Ghoshi, and A. Sen., Phytochemistry, 25,
2117–2122 (1986).