Note

Isolation of Lectins by Affinity Chromatography with Porcine Plasma Proteins Immobilized on Agarose

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To develop a convenient method to isolate lectins, we prepared an affinity gel by coupling plasma proteins with agarose beads under conditions where the pH did not exceed 7.5. The validity of the use of this affinity gel in combination with elution using a hapten saccharide was confirmed by isolation of concanavalin A from Jack bean meal. Successful application of the method was demonstrated by isolation of two novel vegetable lectins from udo (Aralia cordata) and wasabi (Wasabia japonica). The method would be useful to isolate new lectins from various sources including plant and animal tissues.

Key words: Aralia cordata; udo; Wasabia japonica; wasabi; lectin

Lectins are a class of proteins that bind to a carbohydrate moiety of glycoconjugates.1 The biological activities elicited by lectins include cell agglutination, mitosis, toxicity, and cell growth inhibition. Lectins have been used to detect physiological and pathological changes in constituents of normal and diseased tissues and body fluids.2-4 Lectin-based separation methods to prepare specific glycoproteins and specific cell populations have also been widely described.5-7) Recently, we reported that rice bran agglutinin and a lectin from the edible mushroom Boletopsis leucome-lus induced apoptosis in tumor cells.8,9) These cytotoxic lectins could be the subject of clinical trials. In view of the potential usefulness of lectins, further development of methods to provide lectins easily is encouraged.

In this work, we prepared an affinity gel by coupling porcine plasma proteins with agarose beads using the conventional method with a slight modification. Animal blood plasma contains glycoproteins with a variety of carbohydrate chains10 and porcine or bovine blood is easily obtainable in large amounts from a local slaughterhouse. We expected that elution with various kinds of hapten saccharides would provide various lectins with corresponding carbohydrate specificity. By using this method we could isolate lectins from two kinds of vegetables, udo (Aralia cordata) and wasabi (Wasabia japonica). The vegetables were purchased from a local market. Jack bean meal was from Sigma Aldrich Japan (Tokyo, Japan). p-Nitrophenyl-1-thio-α-D-mannoside, β-D-galactoside, β-N-acetyl-D-glucosaminide, and β-г-glucoside were prepared as described previously.10 Sepharose 4B and a Protein Assay kit were from Amersham Biosciences K. K. (Tokyo, Japan) and Nippon Bio-Rad Laboratories (Tokyo, Japan), respectively.

Porcine blood was obtained from a local slaughterhouse. To 200 ml of porcine blood was added 40 ml of 3.8% trisodium citrate to prevent coagulation, and plasma was separated by centrifugation at 11,000 g for 10 min. To 100 ml of porcine plasma (pH 7.4) thus obtained was added 100 g of Sepharose 4B activated with CNBr according to the method described previously except that sodium bicarbonate (pH 8.0) was not used during coupling. The mixture was incubated at 4°C for 18 h. The gel was then blocked with 1 M monoethanolamine-HCl at pH 7.4 and 4°C, and washed exhaustively with phosphate-buffered saline, pH 7.4 (PBS). To confirm the validity of the prepared affinity gel, we tested if it can be used to isolate concanavalin A from Jack bean meal. One hundred g of Jack bean meal was extracted with 200 ml of PBS. The extract was centrifuged at 15,000 g for 10 min. To the supernatant was added ammonium sulfate to give 70% saturation, and the precipitated proteins were separated by centrifugation at 15,000 g for 10 min. The precipitates were dissolved

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Abbreviations: PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
in PBS, and the supernatant separated by centrifugation at 20,000×g for 10 min was dialyzed against PBS, and the non-diffusible protein fraction was centrifuged at 20,000×g for 10 min. The supernatant of this protein fraction was put onto the affinity column prepared as above. Elution with 0.5 M methyl α-D-mannoside yielded a protein with a molecular mass of about 30 kDa to give a single band upon sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) (not shown). The microsequence analysis using an Applied Biosystems 492A gas-phase sequencer as described previously revealed its amino-terminal sequence as A-D-T-I-V-A-V-E-L-D-T-Y-P-N-T. and this was identical to that of concanavalin A.13

For application of this method to isolate a lectin from udo, 100 g of udo stalks was homogenized and the protein fraction was prepared as described above. The udo protein fraction was then put through affinity chromatography. After exhaustive washing with PBS, a sequential elution with 0.2 M lactose, 0.25 M N-acetylglucosamine, and 0.5 M methyl α-D-mannoside was done. Elution with methyl α-D-mannoside afforded a 64 kDa protein on SDS-PAGE with 12% gel (Fig. 1). Comparison of the electrophoretic mobility of the 64 kDa protein under reducing and non-reducing conditions by SDS-PAGE suggested that the protein is 200 kDa in its native form, composed of three disulfide-linked subunits of 64 kDa (Fig. 2). The yield was about 5 mg from 100 g of the raw vegetable. The amino acid composition of the protein as found by the method described previously is listed in Table 1.

The partial N-terminal amino acid sequence was found to be A-N-Y-P-T-V-S-K-T-I-D-G-N-G-. Database searches using an FASTA program indicated that the protein is a novel one. The udo 200 kDa protein showed hemagglutinating activity at concentrations as low as 5 ng/ml (not shown), when examined using a 0.5% suspension of rabbit erythrocytes, confirming that the protein is a lectin. The agglutination was not inhibited by the hapten saccharides tested including 0.2 M lactose, 0.5 M methyl α-D-mannoside, 0.25 M N-acetyl-D-glucosamine, 0.2 M maltose, and 0.2 M D-galactose. The reason for the failure of inhibition by 0.5 M methyl α-D-mannoside is unknown at present. It is speculated that rabbit erythrocytes may contain sugar chains with stronger affinity than porcine blood glycoproteins. Another explanation may be that rabbit erythrocytes contain much more condensed clusters of saccharides responsible for hemagglutinating activity of the lectin.

To examine the interaction of udo lectin with saccharides, equilibrium dialysis was done by the method described previously. Briefly, the lectin solution (50 μl) was dialyzed with 50 μl of ligand solution containing 500 pmol each of p-nitrophenyl 1-thio-α-D-mannoside, β-D-galactoside, β-N-acetyl-D-glucosaminide, and β-D-glucoside in a dialysis cell prepared from plastic microtubes and a dialysis
membrane. After 24 h of dialysis at 5°C, the concentrations of each saccharide in the ligand solution were measured by HPLC as described previously.\(^\text{14}\) The binding constant was calculated from the equation, 
\[ K_a = [L_s] /[L][S], \]
where [L], [S] are the concentrations of bound ligand and free ligand, respectively. [L] represents the concentration of binding sites of the lectin estimated with the assumption that the lectin has a single binding site per polypeptide with a molecular mass measured by SDS-PAGE under reducing conditions.

The result indicated that udo lectin interacted with \( p \)-nitrophenyl 1-thio-\( \alpha \)-d-mannoside with a dissociation constant of 68 \( \mu \)M. No affinity of udo lectin for other \( p \)-nitrophenyl thioglycosides tested was demonstrated by this method. Thus, the udo 200 kDa lectin appears to belong to a mannose-specific lectin family.

When the wasabi rhizomes extract was similarly examined, elution with 0.2 M lactose from the affinity column yielded a 32 kDa protein as detected by SDS-PAGE with a 12% gel (Fig. 2). Comparison of electrophoretic mobility under reducing and non-reducing conditions indicated that the protein is a single polypeptide without subunits linked by a disulfide bond (Fig. 2). The yield was about 3 mg from 100 g of the raw vegetable. The amino acid composition of this protein is listed in Table 1.

The partial N-terminal amino acid sequence was found to be E-T-Y-V-L-Y-K-E-R-P-S-X-K-I-V-T-I-T-S-F-, and the result of a computer search indicated that the protein is a hitherto undescribed one. Its hemagglutination activity confirmed that the protein is a lectin (not shown). The minimum concentration required for the activity was about 50 ng/ml. The agglutination of rabbit erythrocytes was partially inhibited with 0.2 M lactose, but not with 0.5 M methyl \( \alpha \)-d-mannoside, 0.25 M N-acetyl-d-glucosamine, 0.2 M maltose, or 0.2 M d-galactose.

The results of the equilibrium dialysis method indicated that the dissociation constant of the lectin for \( p \)-nitrophenyl thio-\( \beta \)-D-galactoside was 1.3 nm. No affinity of the wasabi lectin for other \( p \)-nitrophenyl thioglycosides tested was demonstrated by this method. Thus, the wasabi 32 kDa lectin appears to belong to a galactose-specific lectin family and its low affinity for the \( \beta \)-D-galactosides tested suggests that the lectin recognizes an as yet unidentified galactose-containing structure.

Some galactose-specific lectins are known to bind to agarose.\(^\text{15}\) However, the wasabi lectin was not retained on a plain Sepharose 4B column (not shown), suggesting its lack of affinity for agarose itself.

Thus, we could isolate two novel vegetable lectins with different carbohydrate specificity by affinity chromatography with immobilized porcine plasma proteins using elution with different carbohydrate solutions. This inexpensive and effective method would facilitate isolation of potentially important lectins from dormant biological resources.

References


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