J. Biochem. **127**, 129–135 (2000)

A Chimeric Lectin Formed from *Bauhinia purpurea* Lectin and *Lens culinaris* Lectin Recognizes a Unique Carbohydrate Structure[1]

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Received September 2, 1999; accepted October 25, 1999

Lectins are carbohydrate-binding proteins widely used in biochemical, immunochemical, and histochemical studies. *Bauhinia purpurea* lectin (BPA) is a leguminous lectin with an affinity for galactose and lactose. Nine amino acids, DTWPNTEWS, corresponding to the amino acid sequence from aspartic acid-135 to serine-143 in the primary structure of BPA were replaced with the corresponding amino acid residues from the mannose-binding *Lens culinaris* lectin (LCA), and the chimeric lectin obtained was expressed in *Escherichia coli* cells. The carbohydrate-binding specificity of the recombinant chimeric lectin was investigated in detail by comparing the elution profiles of various glycopeptides and oligosaccharides with defined carbohydate structures from immobilized lectin columns. Glycopeptides carrying three constitutive carbohydrate sequences of Galβ1-3GalNAc-Ser/Thr and a complex-type biantennary glycopeptide, which show a high affinity for BPA or LCA, were shown to have no affinity for the chimeric lectin. In contrast, hybrid-type and high mannose-type glycopeptides with a Manα1-6-(Manα1-3)Manα1-6Man sequence were found to have a moderate affinity for the chimeric lectin. This result demonstrates that a novel type of lectin with a unique carbohydrate-binding specificity can be constructed from BPA by substituting several amino acid residues in its metal-binding region with other amino acid residues. Additional lectin(s) with distinctly different carbohydrate-binding specificities will provide a powerful tool for many studies.

Key words: *Bauhinia purpurea* lectin, chimera, lectin, *Lens culinaris* lectin, specificity.

Bauhinia purpurea lectin (BPA) is specific for galactose and lactose, and preferentially binds to Galβ1-3GalNAc (*1*). We have already cloned a cDNA coding BPA and determined its complete amino acid sequence (*2*). Further, we purified a nonapeptide involved in the carbohydrate-binding of BPA and determined its amino acid sequence to be DTWPNTEWS (*3*). The amino acid sequence of this peptide was found to be in the metal-binding region of BPA. Also in the same paper, we reported that a synthetic nonapeptide, DTWPNTEWS, binds to lactose in the presence of calcium ions. This indicates that the nonapeptide is actually derived from part of the carbohydrate-binding site of BPA, and therefore, also confirms directly the carbohydrate-binding specificity of BPA. Moreover, we isolated peptide fragments with affinities for specific carbohydrates from several plant lectins, including *Lotus tetragonolobus* lectin, *Ulex europaeus* lectin-I, *Ulex europaeus* lectin-II, *Labrunum alpinum* lectin-I (*4*), *Cytisus sessilifolius* lectin-I (*5*), and *Cytisus scoparius* lectin-II (*6*). Their amino acid sequences suggest they share a homologous domain that interacts with metals as well as with specific carbohydrate chains in legume lectins. As described in our previous paper (*7*), we constructed a chimeric lectin gene using a cDNA clone coding BPA whose nonapeptide sequence, DTWPNTEWS, was replaced by the corresponding region of the mannose-binding *Lens culinaris* lectin. The chimeric lectin, BPA/LCA, when expressed in *E. coli* cells was found to bind α-mannosyl-bovine serum albumins in a manner that is inhibited by mannose.

Originally, it was believed that the specificity of lectins could be described in terms of the monosaccharides that best inhibited the lectin-induced agglutination of cells. In fact, lectin-reactive monosaccharides are divided into four classes based on their configuration at C-3 and C-4 of the pyranose ring. However, it has been found that the detailed carbohydrate-binding specificities differ among the same class of lectins.

In the present study, we determined the detailed carbohydrate-binding specificity of a chimeric lectin, BPA/LCA, using lectin affinity chromatography. Although both LCA and the chimeric lectin bind to mannose, the chimeric lectin recognizes a much more distinct carbohydrate sequence compared with LCA.

[1] This work was supported by Grants-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan (08672502, 10672038), Ministry of Health and Welfare, the Hayashi Memorial Foundation for Female Natural Scientists and an Esso Research Grant for Women.

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Abbreviations: BPA, *Bauhinia purpurea* lectin; LCA, *Lens culinaris* lectin; Gal, galactose; GalNAc, *N*-acetylgalactosamine; Man, mannose; CB-II, an N-terminal glycopeptide of human erythrocyte glycophorin A.

MATERIALS AND METHODS

Materials—Galactosylated bovine serum albumin (Gal-BSA), mannosylated BSA (Man-BSA), *N*-acetylgalactosaminylated BSA (GalNAc-BSA), and *N*-acetylglucosaminylated BSA (GlcNAc-BSA) were purchased from Sigma (St. Louis, MO, USA). Iodogen as an iodination reagent was from Pierce (Rockford, IL, USA). All other reagents were of analytical grade.

Construction of Expression Plasmid and the Expression of the Chimeric Lectin Formed From BPA and LCA in E. coli Cells—The coding region of the BPA cDNA flanked by artificial sites for *Bam*HI and *Eco*RI was amplified by polymerase chain reaction (PCR) as described previously (*7*). A cDNA coding the NH_2-terminal fragment of BPA, extending from nucleotide 85 to 493 of the BPA coding region, and flanked by artificial sites for *Nde*I and *Fun*4HI, was amplified by PCR using primers 5′-CGTGGATCCACAAGCTCAACCTTA-3′ (BamHI primer) and 5′-CGCTTGCTGCATTGTAGAAAGTGTCAAATTCAACAGCAAC-3′ (Fun4HI primer-a). A cDNA coding the COOH-terminal half of BPA from nucleotide 514 to 870 flanked by artificial sites for *Fun*4HI and *Bam*HI was also constructed by PCR using primers 5′-CGCAGCATGGGACCTACGTTATCCACAT-3′ (Fun4HI primer-b) and 5′-CCCGAATTCTTACATACTGGAATAAGAG-3′ (EcoRI primer). These fragments were digested with *Fun*4HI and then ligated. The ligated DNA thus prepared was further amplified by PCR using *Bam*HI and *Eco*RI primers. Finally, after digestion with *Bam*HI and *Eco*RI, the DNA fragment generated by PCR was inserted between the *Eco*RI and *Bam*HI sites of pGEX-2T (Pharmacia, Uppsala, Sweden) to yield a plasmid, pGEX-BPA/LCA. The constructed plasmid was introduced into *E. coli* strain JM109 cells. The JM109 cells containing plasmid pGEX-BPA/LCA were grown to mid log phase at 37°C in 2 × YT medium containing 5 mM each $CaCl_2$ and $MnCl_2$, and then induced by adding isopropyl-β-thiogalactoside. The *E. coli* cells harvested by centrifugation were suspended in 50 mM Tris/HCl, pH 7.4 and lysed with a sonifier (Branson, USA) at level 7 for 10 pulses, each for 30 seconds. After centrifugation at 12,000 ×*g* for 10 min, the supernatant was applied to a column (1.2 cm × 5 cm) of Glutathione-Sepharose 4B (Pharmacia). After washing the column with 50 mM Tris/HCl, pH 7.4, a lectin–glutathione–S-transferase fusion protein was eluted with 50 mM Tris/HCl, pH 7.4, containing 10 mM glutathione (reduced form). The recombinant fusion protein (10 mg) was then applied to a column of maltose-Sepharose to recover a recombinant lectin with sugar-binding activity. The bound fraction, eluted with 50 mM Tris/HCl, pH 7.4, containing 0.1 M maltose, was collected and used for the following experiments. The obtained product had an approximate molecular weight of 60,000 as demonstrated by polyacrylamide gel electrophoresis in the presence of sodium dodesyl sulfate (*8*).

Affinity Chromatography on Immobilized Recombinant Chimeric Lectin BPA/LCA—The purified recombinant chimeric lectin BPA/LCA was coupled to formyl-cellulofine (Seikagaku Kogyo, Tokyo) according to the method of Jentoft and Dearborn (*9*) at a concentration of 5 mg/ml gels. Affinity chromatography was performed at 22°C. Radiolabeled oligosaccharide or glycopeptide samples (2,000 cpm in 100 μl) were loaded onto a column of BPA/LCA-cellulofine (0.6 × 4.0 cm) equilibrated with 50 mM Tris/HCl, pH 6.8. The column was eluted with the same buffer and then with 50 mM glycine/HCl, pH 3.0. Fractions (0.2 ml) were collected at a flow rate of 0.6 ml/h. The radioactivity of each fraction was measured with a liquid scintillation counter.

Glycopeptides and Oligosaccharides Used for Affinity Chromatography—Figure 1 shows the structures of glycopeptides and oligosaccharides used in this study. They were prepared as previously described and their structures were confirmed by compositional analyses, methylation analyses, and sequential glycosidase digestions (*10, 11*). Various Asn-linked glycopeptides were prepared by repeated proteinase digestion of the corresponding glycoproteins (*12, 13*). Oligosaccharides were released from glycopeptides by hydrazinolysis as described previously (*13, 14*). Fractionation of Asn-linked oligosaccharides was generally carried out as in the following example for transferrin oligosaccharides: Asn-linked complex-type oligosaccharides from human serum transferrin were applied to a column (1.0 × 5.0 cm) of Con A–Sepharose (Pharmacia) equilibrated with 50 mM sodium acetate buffer, pH 6.0, containing 0.15 M NaCl, 1 mM $CaCl_2$, and 1 mM $MnCl_2$. After elution of the triantennary oligosaccharides with the same buffer, biantennary oligosaccharides retained by the column were eluted with the same buffer containing 0.1 M methyl-α-mannoside. After the removal of sialic acid residues by mild acid hydrolysis (0.1 M HCl, 80°C, 30 min), the Con A–unbound fraction was loaded onto an L-PHA-Sepharose column (0.5 × 12.0 cm). The column was eluted with 10 mM sodium phosphate buffer, pH 7.4, containing 0.15 M NaCl. The structures of L-PHA–retarded and unretarded fractions were triantennary oligosaccharides with outer α-mannosyl residues substituted at C-2 and C-6, and oligosaccharides similar to those at C-2 and C-4, respectively (*15*). The Con A–bound fraction was further fractionated by anion-exchange chromatography on a column (1.5 × 16.0 cm) of DEAE-Sephadex A-25 (Pharmacia). The column was eluted with 2 mM Tris/HCl, pH 7.4, and then with a linear gradient of NaCl (0–0.2 M) in the same buffer to obtain biantennary monosialo- and disialo-oligosaccharides. Their carbohydrate structures were confirmed by gel permeation chromatography on a column of Bio-Gel P-4 (Bio-Rad Laboratories, CA, USA) before and after the sequential removal of non-reducing terminal saccharides.

Hybrid-type glycopeptides GP-I, GP-II-A, GP-II-B, GP-IV, and GP-V from ovalbumin were obtained according to Yamashita *et al.* (*16*) and Tai *et al.* (*17*). Asialo-tetraantennary oligosaccharides from human α1-acid glycoprotein were prepared by the method of Yoshima *et al.* (*18*). Specific activities of radiolabeled glycopeptides and oligosaccharides were about 7.5×10^{10} and 8.9×10^{10} dpm/mmol, respectively.

An N-terminal glycopeptide of human erythrocyte glycophorin A (CB-II) was prepared from tryptic fragment T1 according to the method of Prohaska *et al.* involving cyanogen bromide cleavage (*19*), and acetylated with [^{14}C]acetic anhydride (2 mCi/mmol, New England Nuclear, Boston, MA, USA). Sialic acid residues of [^{14}C]CB-II were removed by mild acid hydrolysis in 50 mM HCl at 80°C for 1 h. Asialo, agalacto-[^{14}C]CB-II was obtained after Smith periodate degradation of asialo-[^{14}C]CB-II (*20*). The second round Smith periodate degradation of asialo, agalacto-[^{14}C]CB-II gave [^{14}C]CB-II peptide without sugar moieties. The α2-3 linked sialic acid residues of [^{14}C]CB-II were removed by α2-3 sial-

idase from *Salmonella typhimurium* (Sigma). The removal of the sialic acid residues was confirmed by the elution position from anion-exchange chromatography on a Mono Q 5/5 column (Pharmacia). The structures of the derivatives of CB-II are summarized in Fig. 2.

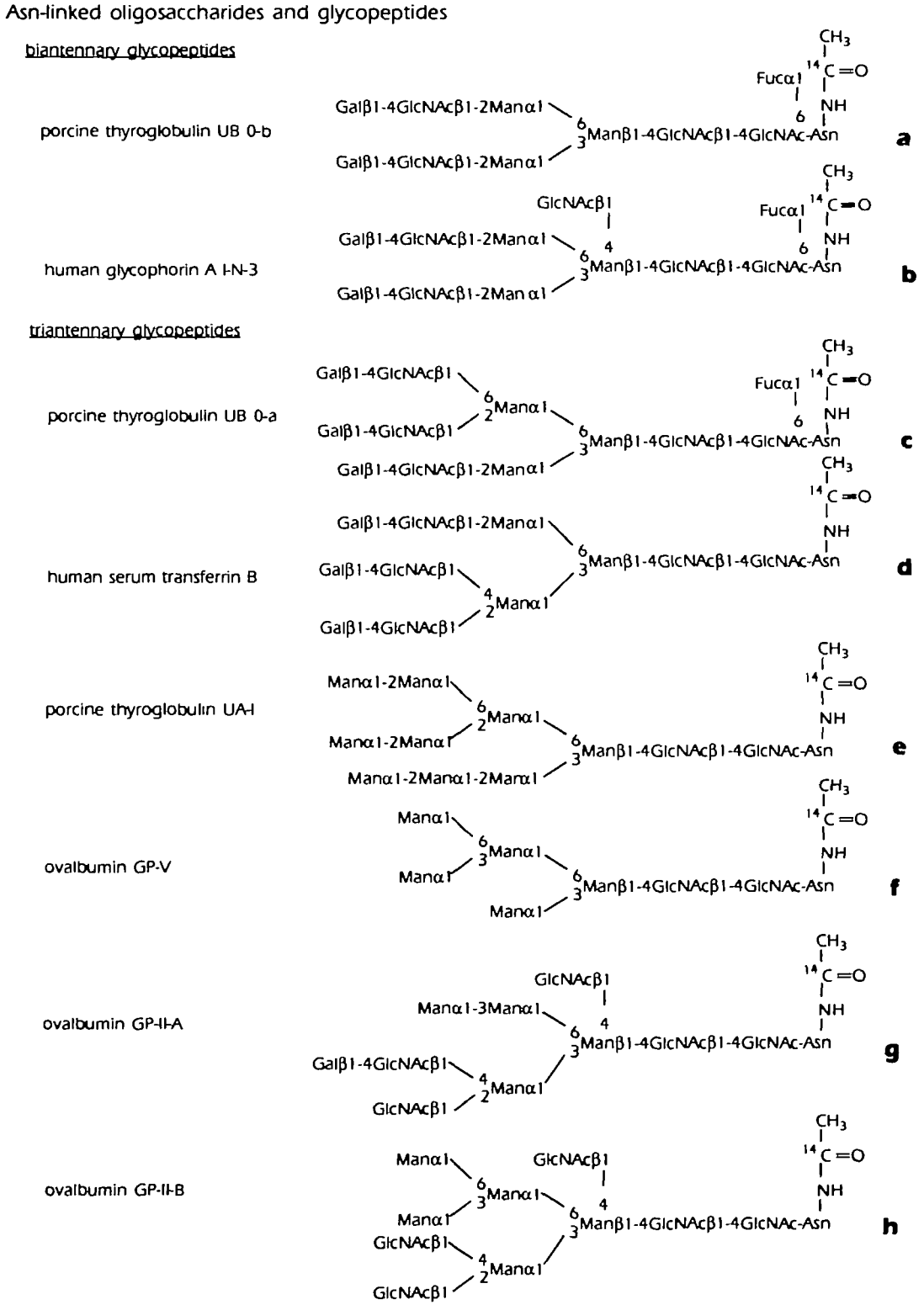

Fig. 1 (continued on next page)

RESULTS AND DISCUSSION

Construction and Expression of Chimeric Lectin BPA/LCA—Bauhinia purpurea lectin (BPA) is a β-galactoside-specific legume lectin. Previously, we showed that the nonapeptide, DTWPNTEWS, of BPA, which is part of the metal-binding region, is involved in its carbohydrate-binding (*3*). A chimeric BPA/LCA lectin, in which the amino acid residues in the nonapeptide sequence of BPA are replaced by those corresponding to the region of the mannose-binding *Lens culinaris* lectin (LCA), DTFYNAAW, was found to bind to α-mannosyl-BSA instead of β-galactosyl-BSA (*7*). This indicates that the carbohydrate-binding specificity of BPA can be altered by substituting seven amino acid residues in part of the metal-binding region with the corresponding residues in LCA (*7*). In order to obtain a large amount of the recombinant chimeric BPA/LCA lectin, we constructed a BPA/LCA chimeric lectin gene by PCR and ligated it to the expression plasmid pGEX-2T. The obtained recombinant chimeric lectin expressed in *E. coli* cells as a glutathione-S-transferase (GST, 26 kDa) fusion protein, was purified to homogeneity by chromatography on a column of glutathione-Sepharose 4B, and shown to migrate as a 60 kDa band in SDS–polyacrylamide gels (Fig. 3). The recombinant chimeric lectin BPA/LCA with carbohydrate-binding activity was further purified by chromatography on a maltose-Sepharose column. GST-fused chimeric lectin

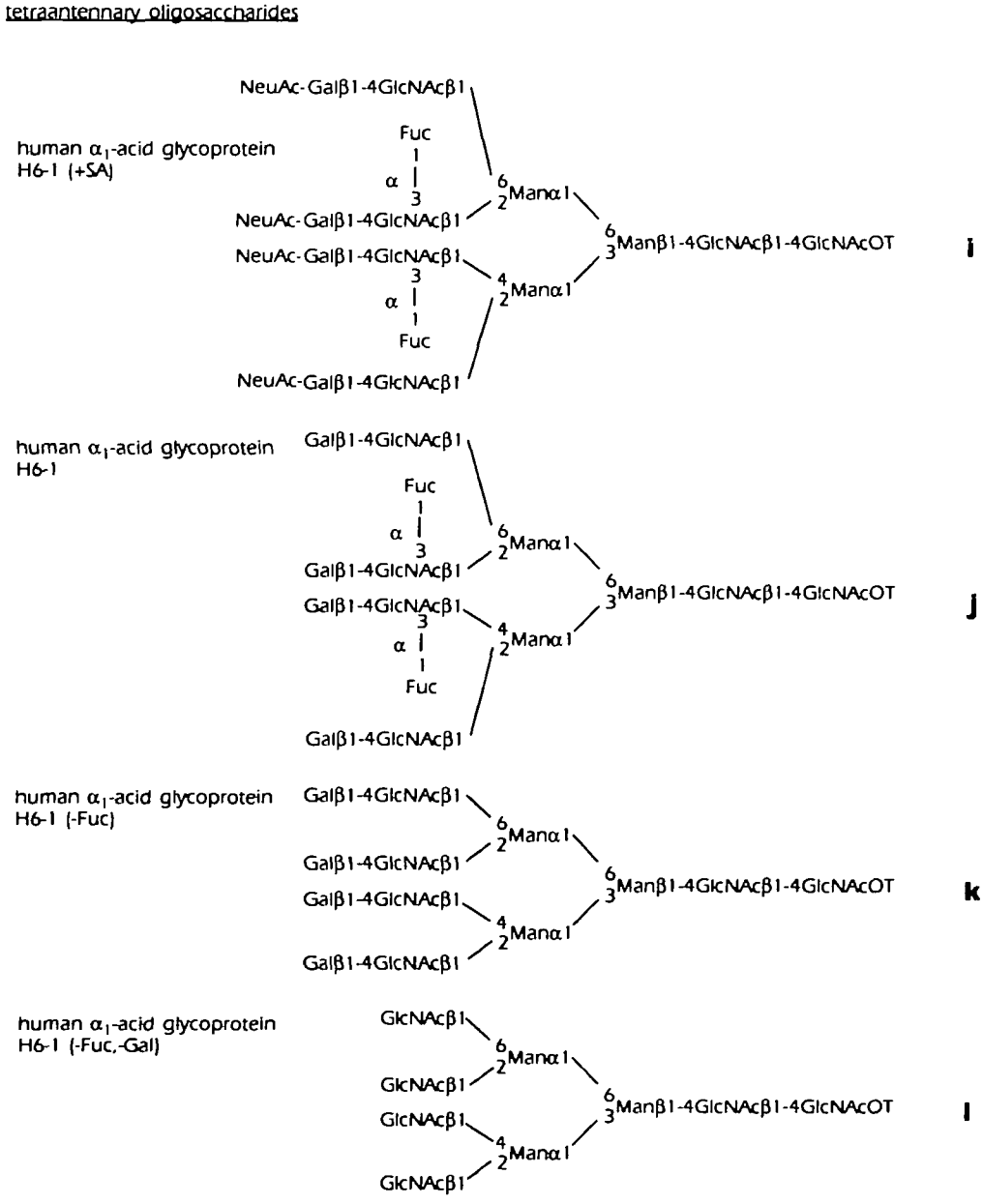

Fig. 1. **Structures of the *N*-linked glycopeptides and oligosaccharides used in this study.** Details are described in "MATERIALS AND METHODS."

BPA/LCA bound to ^{125}I-labeled Man-BSA but not to ^{125}I-labeled Gal-BSA, GlcNAc-BSA, or GalNAc-BSA (data not shown). To examine the carbohydrate-binding specificity of the recombinant lectin in detail, affinity chromatography on a column of immobilized recombinant lectin (~5 mg/ml gel) was performed. A desialylated, biantennary complex-type glycopeptide containing a fucose residue attached to the C-6 position of the innermost *N*-acetylglucosamine (Fig. 1, a) is known to bind to an LCA-Sepharose column (*21, 22*). An asialo CB-II glycopeptide, which contains three Galβ1-3GalNAc sugar moieties at the Ser-Ser-Thr sequence (Fig. 2, o), has been shown to bind to a column of BPA-Sepharose (*10*). These two glycopeptides were applied to the immobilized BPA/LCA chimeric lectin column, and neither was recovered in the void volume without retardation (Fig. 4a). Since the chimeric lectin binds to mannose as reported previously (*7*), several oligosaccharides and glycopeptides with mannose residue(s) at their nonreducing termini were tested in the same manner. Figures 1 and 2 show oligosaccharides and glycopeptides applied to the chimeric lectin column in this study. Among these sugar chains, a hybrid-type glycopeptide GP-II-B of ovalbumin (Fig. 1, h) showed significant retardation on the BPA/LCA-Sepharose column (Fig. 4b). A GP-I glycopeptide from ovalbumin with a se-quence containing Manα1-6(Manα1-3)-Manα1-6Man at the C-6 position of the core β-mannosyl residue was found to have an affinity for the column (data not shown). A GP-II-A hybrid-type glycopeptide with a similar Manα1-3Manα1-6Man structure at one branch (Fig. 1, g) showed a weaker affinity for the column than GP-II-B (Fig. 4a). A high mannose-type glycopeptide GP-V with the structure of Manα1-6(Manα1-3)Manα1-6Man (Fig. 1, f) had an affinity for the BPA/LCA column and was retarded (Fig. 4b). A high mannose-type glycopeptide (UA-I) composed of nine mannose residues and two *N*-acetylglucosamine residues (Fig. 1, e) showed decreased affinity for the chimeric BPA/LCA column. Other oligosaccharides and glycopeptides listed in Figs. 1 and 2 showed no affinity for the chimeric lectin column (data not shown). These data indicate that the sequence Manα1-6(Manα1-3)Manα1-6Man at the nonreducing terminus is essential for strong binding to the BPA/LCA chimeric lectin, and that substitution of the nonreducing terminal mannose with mannose residues *via* α1-2 linkages decreases the affinity.

Lectins have been extensively used in studies on glycoconjugates, especially for the histochemical detection of cell surface carbohydrate chains, staining, and the structural estimation of glycoproteins on Western blotted membranes

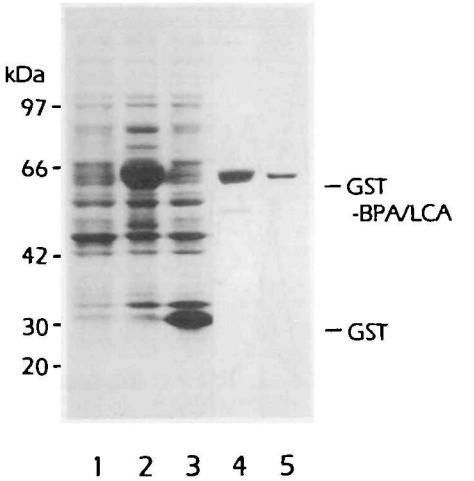

Fig. 3. **Expression of the recombinant BPA/LCA chimeric lectin in *E. coli* cells.** Protein samples were analyzed by electrophoresis in a 10% SDS–polyacrylamide gel, followed by staining with Coomassie Blue. Lane 1, cell lysate of *E. coli* cells having pGEX-BPA/LCA without induction; lane 2, cell lysate of *E. coli* cells having pGEX-BPA/LCA after the induction with 1 mM IPTG; lane 3, cell lysate of *E. coli* cells having pGEX-2T after the induction with 1 mM IPTG; lane 4, the chimeric lectin purified on a column of Glutathione-Sepharose 4B; lane 5, the chimeric lectin further purified on a column of maltose-Sepharose.

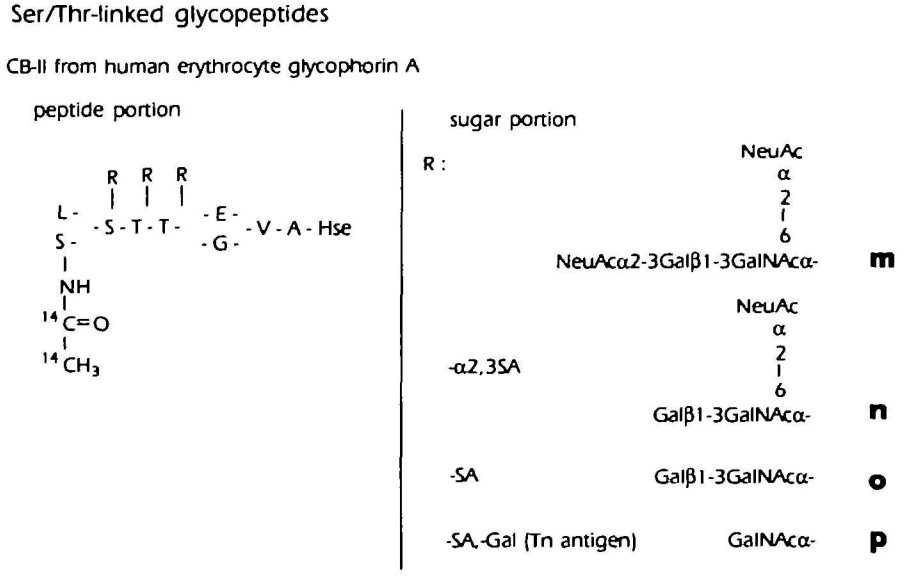

Fig. 2. **Structures of the *O*-linked glycopeptides used in this study.** Octapeptides derived from the amino terminal portion of human glycophorin A (CB-II) were subjected to sequential chemical and enzymatic trimming of carbohydrate chains, as described in the "MATERIALS AND METHODS." The amino acid sequence is based on the data reported by Marchesi and coworkers (*23*).

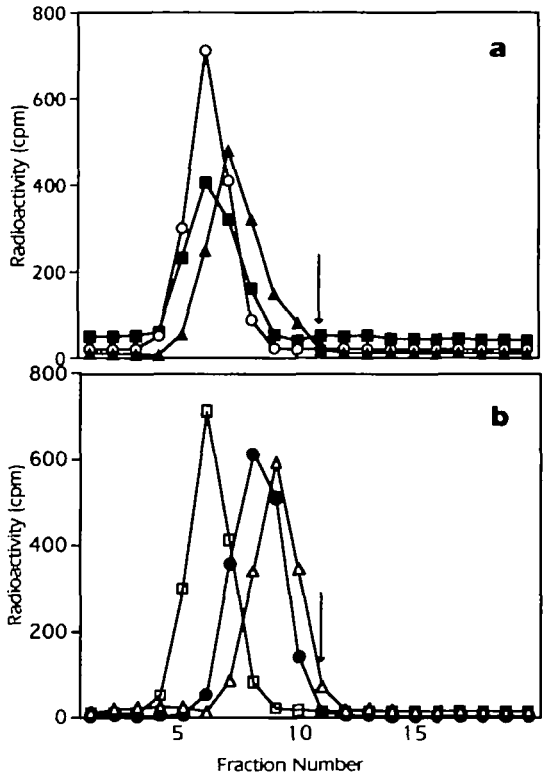

Fig. 4. **Elution profiles of glycopeptides on a column of immobilized recombinant chimeric BPA/LCA lectin.** (a) Open circles, asialo-biantennary complex-type glycopeptide from porcine thyroglobulin (UB 0-b); another asialo-biantennary and triantennary complex-type carbohydrate chains eluted at similar positions to UB 0-b. Closed squares, asialo-CB-II from human glycophorin A (glycopeptides CB-II-SA). Other CB-II and asialo-, agalacto-CB-II (CB-II and CB-II-SA, -Gal) eluted at similar positions to CB-II-SA: Closed triangles, hybrid-type glycopeptide from ovalbumin (GP-II-A). Arrow indicates a switch in the elution buffer to a buffer of 50 mM glycine/HCl, pH 3.0. (b) Open triangles, galactosylated hybrid-type glycopeptide GP-II-B from ovalbumin (GP-II-B). Closeds circles, high mannose-type glycopeptide GP-V (GP-V). Open squares, high mannose-type glycopeptide from porcine thyroglobulin (UA-I). The arrow indicates a switch in the elution buffer to a buffer containing 50 mM glycine/HCl, pH 3.0.

or glycolipids on thin layer chromatography plates, separation of cells with different cell surface carbohydrate chains, and the isolation and fractionation of glycoproteins. If additional lectin(s) with a distinct carbohydrate-binding specificity is found, this technique would be a more powerful tool in its study. In this paper, we demonstrate that a different carbohydrate-binding specificity is introduced into BPA lectin by substituting the amino acid residues of a nonapeptide in BPA, which was presumed to be involved in its carbohydrate-binding, with those from the corresponding sequence of LCA lectin. This also indicates that other recombinant lectins with distinct carbohydrate-binding specificities could be prepared by changing the amino acid sequences of part of the metal-binding region of BPA lectin.

Leguminous lectins form a family of proteins with homologous sequences and similar three-dimensional structures. The crystal structures of some of these have been solved, as well as their complexes with monosaccherides or oligosaccharides. X-ray studies have shown that the folding of the polypeptide chains in the region of the carbohydrate-binding site is also similar. Carbohydrate-binding sites in these lectins consist in 2 conserved amino acids on β-pleated sheets and 2 loops. One of these loops contains a transition metal and keeps the amino acid residues of the carbohydrate-binding site into required positions. The mutated residues introduced into the BPA cDNA in this study correspond to one of these two loops. This indicates that the different amino acid sequences that have been introduced form only a part of the carbohydrate-binding pocket that consists of a part of both LCA and BPA. This may explain the difference in the details of the carbohydrate-binding specificity of the chimeric lectin BPA/LCA compared with those of BPA or LCA.

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