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Carbohydrate Binding Specificities of Several Poly-*N*-acetyllactosamine-binding Lectins

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The structural requirements for the interaction of the Asn-linked poly-*N*-acetyllactosamine-type oligosaccharide moieties of glycoproteins with various *N*-acetylglucosamine-binding lectins were investigated by means of affinity chromatography on immobilized lectin-Sepharose columns.

High molecular weight glycopeptides containing poly-*N*-acetyllactosamine-type oligosaccharides obtained by Pronase digestion of human erythrocyte ghosts were treated with 0.1 M trifluoroacetic acid at 100°C for 40 min and then several oligosaccharide fragments were purified with an amino-bonded silica column. Among these oligosaccharide fragments, trisaccharide Galβ1-4GlcNAcβ1-6Galol bound to the wheat germ agglutinin (WGA)- and pokeweed mitogen (PWM)-Sepharose columns, and also showed affinity to the *Datura stramonium* agglutinin (DSA)-, *Lycopersicon esculentum* (tomato) agglutinin- and *Solanum tuberosum* (potato) agglutinin-Sepharose columns. Pentasaccharide Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galol showed weaker affinity to the WGA- and PWM-Sepharose columns, compared to the trisaccharide. Trisaccharide GlcNAcβ1-3(GlcNAcβ1-6)Galol showed weak affinity to the WGA-Sepharose column and did not show any affinity to the other lectin-Sepharose columns. Hexasaccharide Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcol bound only to the DSA-Sepharose column, indicating that only DSA does not require a GlcNAcβ(1-6)- linkage for interaction.

Lectins are cell-agglutinating proteins of non-immune origin that bind to carbohydrates specifically without modifying them [1]. So far, more than a hundred plant lectins have been isolated, and are widely used for the separation of oligosaccharides and glycopeptides [2-7], staining and structural characterization of electrophoretically separated membrane

Abbreviations: HPLC; high performance liquid chromatography; WGA, wheat germ agglutinin; PWM, pokeweed mitogen; DSA, *Datura stramonium* agglutinin; LEA, *Lycopersicon esculentum* (tomato) agglutinin; STA, *Solanum tuberosum* (potato) agglutinin; EVA, *Erythrina variegata* agglutinin; PBS, 10 mM sodium phosphate buffer, pH 7.2, containing 0.15 M NaCl; Galol, galactitol; GlcNAcol, *N*-acetylglucosaminitol.

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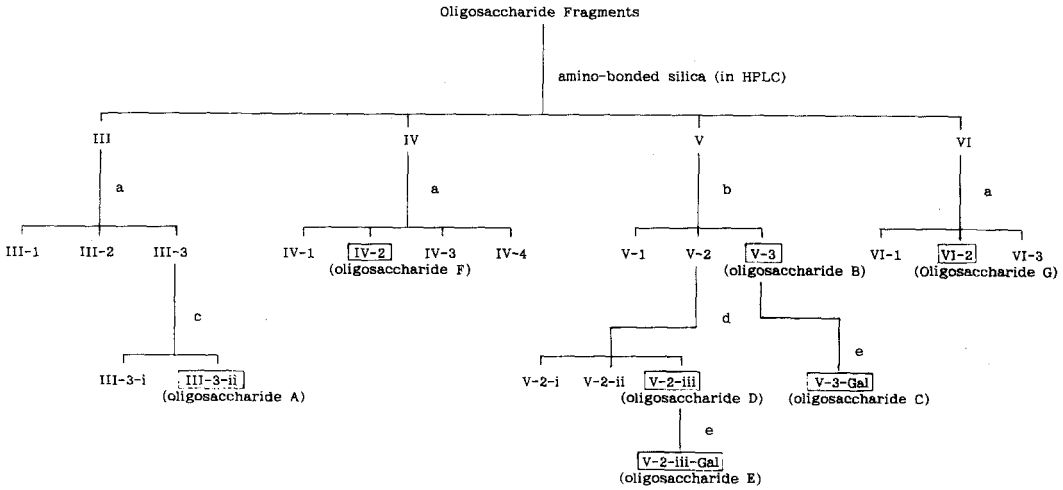

Figure 1. Separation of the oligosaccharides on lectin-affinity columns. Fraction III, IV or VI from amino-bonded silica column was applied to a WGA-lectin-affinity HPLC column (a), and fraction V from amino-bonded silica column to an EVA-Sepharose column (b). Fraction III-3 was applied to a RCA-Sepharose column (c) and fraction V-2 was further applied to an LEA-Sepharose column (d). Fractions V-3 and V-2-iii were treated with β-galactosidase (e) at 37°C for 18 h. The fractions were named oligosaccharides A-G, as indicated in the Figure.

glycoconjugates [8-10], identification of immunocyte subsets [11-12] and cells in various differentiation stages [13], and so on. In most cases, membrane glycoconjugates on cell surfaces are very difficult to isolate in sufficient quantities, so plant lectins are useful for their structural characterization.

Recently, a novel type of carbohydrate chain, the so-called poly-*N*-acetyllactosamine-type oligosaccharide, has been described [14-20]. Its side chains have a characteristic structure composed of *N*-acetyllactosamine (Galβ1-4GlcNAc) repeating units. The carbohydrate moiety of human erythrocyte band 3 glycoprotein contains an I-antigenic structure, which is a branched poly-*N*-acetyllactosaminoglycan containing Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Gal units [14, 15]. Furthermore, it is known that during the development of erythrocytes from the fetal to adult stage, the linear poly-*N*-acetyllactosamine structure (i-antigen), which lacks galactose residues substituted at the C-3,6 positions, is converted to a branched oligosaccharide (I-antigen) [21]. This carbohydrate sequence also occurs in a number of developmentally manifested antigens that are associated with glycolipids and glycoproteins [22-24]. However, the structural characterization of poly-*N*-acetyllactosamine-type oligosaccharides has been quite difficult because the side chains vary as to the number of *N*-acetyllactosamine repeating units and the branching mode. So poly-*N*-acetyllactosamine-binding lectins whose binding specificities have been completely determined may be quite useful.

So far, several poly-*N*-acetyllactosamine-binding lectins have been reported: wheat germ agglutinin (WGA) [25-27], pokeweed mitogen (PWM) [28, 29], *Datura stramonium* agglutinin (DSA) [30, 31], *Lycopersicon esculentum* (tomato) agglutinin (LEA) [19] and *Solanum tuberosum* (potato) agglutinin (STA) [32]. In the present study, we compared and determined the structural requirements for the interaction of these lectins with poly-*N*-acetyllactosamine-type oligosaccharides, using immobilized lectin-Sepharose columns.

Materials and Methods

Materials

Wheat germ agglutinin (WGA), pokeweed mitogen (PWM) and *Ulex europeus* agglutinin II (UEA-II) were purchased from Hohnen Oil Co., Ltd. (Tokyo, Japan). *Datura stramonium* agglutinin (DSA), *Lycopersicon esculentum* (tomato) agglutinin (LEA) and *Solanum tuberosum* (potato) agglutinin (STA) were purchased from BioCarb Chemicals (Lund, Sweden). Lectins were coupled to cyanogen bromide-activated Sepharose 4B by the method of Cuatrecasas and Anfinsen [33]. Sepharose 4B, Sephadex G-25, Sephadex G-50 and Mono Q ion-exchange columns were purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). β-Galactosidase and β-*N*-acetylhexosaminidase from jack bean meal were purchased from Seikagaku Kogyo Co., Ltd. (Tokyo, Japan). Chitin and *N*-acetylglucosamine were from Nakarai Chemicals Ltd. (Kyoto, Japan). Other reagents were of analytical grade.

Carbohydrate Composition and Methylation Analyses

The carbohydrate composition of each oligosaccharide was analysed with a gas chromatograph (Shimadzu GC-9A; Shimadzu Corp., Kyoto, Japan) equipped with a CBP5 column (0.2 mm x 25 m), after hydrolysis with 2 M HCl at 100°C for 3 h and subsequent conversion to the respective alditol acetate.

Methylation of oligosaccharide-alditols was performed as described by Ciucanu and Kerek [34] and the permethylated sugars were purified on a small column of silica gel according to the method of Yamashita *et al.* [35]. The alditol acetates of the partially methylated oligosaccharide alditols were prepared by hydrolysis with 3 M HCl at 80°C for 3 h, followed by reduction with NaB^3H_4 and acetylation as described by Stellner *et al.* [36] and then they were analyzed by gas chromatography mass spectrometry (Shimadzu QP-1000; Shimadzu Corp., Kyoto, Japan) on a column of CBP5 (0.2 mm x 25 m).

Preparation of Oligosaccharides Derived from Poly-N-Acetyllactosamine-type Oligosaccharides through Partial Acid Hydrolysis

About 2.0 g of lyophilized human erythrocyte (blood group O) ghosts were repeatedly digested with Pronase and the resultant high molecular weight glycopeptides containing poly-*N*-acetyllactosamine-type oligosaccharides were isolated by Sephadex G-50 (1.5 x 120 cm) gel filtration, the column having been equilibrated with 185 mM pyridine-acetic acid, pH 6.2. The glycopeptides were cleaved partially with 0.1 M trifluoroacetic acid (TFA) at 100°C for 40 min according to the method of Renkonen *et al.* [27], followed by *N*-

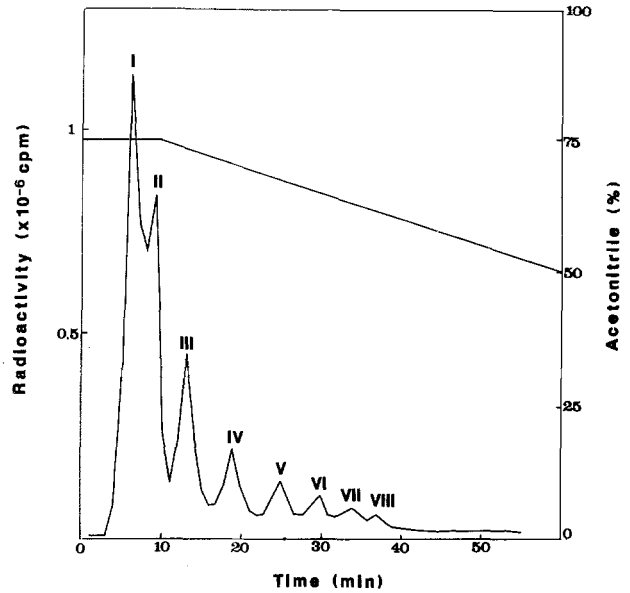

Figure 2. Chromatography of the radioactively labeled oligosaccharide fragments derived from poly-*N*-acetyllactosamine-type oligosaccharides through partial acid hydrolysis on a column of amino-bonded silica. The amino-bonded silica column (0.4 x 25 cm) was eluted at the flow rate of 1.0 ml/min, for 10 min isocratically with 75% acetonitrile, followed by a linear gradient to 50% acetonitrile in 50 min.

acetylation and filtration in water through AG1-X8 (OH^-; 10 ml) and Dowex 50W-X8 (H^+; 10 ml). One twentieth of the oligosaccharides isolated was labeled by reduction with NaB^3H_4, and the remainder was reduced with $NaBH_4$. The reduced oligosaccharides (oligosaccharide fragments, Fig. 1) were then separated on an amino-bonded silica column (Lichrosorb-NH_2, 0.4 x 25 cm) at a flow rate of 1.0 ml/min in water and acetonitrile, for 10 min isocratically with 75% acetonitrile, followed by a linear gradient to 50% acetonitrile in 50 min. The oligosaccharides, III-IV, obtained from the amino-bonded silica column were further purified. Fractions III, IV and VI were separated on a WGA lectin-affinity HPLC column (5 mg/ml gel; Hohnen Oil Co.), which was eluted with 50 mM Tris-HCl, pH 7.2, and then with the same buffer containing 0.2 M *N*-acetylglucosamine, at the flow rate of 0.5 ml/min. Fraction V was separated on an EVA (*Erythrina variegata* agglutinin)-Sepharose column [37], which was eluted with 10 mM sodium phosphate buffer, pH 7.2, containing 0.15 M NaCl (PBS) and then with the same buffer containing 0.2 M lactose, at the flow rate of 4 ml/h. Fraction III-3 and fraction V-2 were further purified on the RCA- and LEA-Sepharose columns, respectively. Then each fraction was desalted on a Bio-Gel P-4 or Bio-Gel P-2 gel filtration column in an HPLC system. The fractions were named oligosaccharides A-G, as shown in Fig. 1. Structural characterization was performed by exo-glycosidase digestion and methylation analysis.

Lectin Affinity Column Chromatography of Oligosaccharides

Each radioactively labeled sample listed in Table 1 was loaded on a lectin column (0.5 x 12 cm) as described above, fractions (0.36 ml) being collected at a flow rate of 4 ml/h. Each fraction was counted in a toluene/Triton scintillation cocktail with a liquid scintillation counter.

Results

Preparation of Oligosaccharides Derived from Poly-N-Acetyllactosamine through Partial Acid Hydrolysis

To compare and determine the essential structures for the retention on the columns of poly-*N*-acetyllactosamine-binding lectins, glycopeptides containing branched poly-*N*-acetyllactosamine-type oligosaccharides prepared by Pronase-digestion of human erythrocyte ghosts were partially hydrolysed with TFA and then oligosaccharide fragments were purified on an amino-bonded silica column. As shown in Fig. 2, fractions I-VIII were obtained, the fraction numbers corresponding to the component carbohydrate residues. As fractions I-VIII were still heterogeneous, each fraction had to be separated on a WGA-lectin-affinity HPLC column or other lectin-Sepharose columns (Fig. 1). Eventually, oligosaccharides A-G were obtained and used in this study as follows.

Oligosaccharide A

Oligosaccharide A was a trisaccharide (Fig. 3-A-a), because it corresponded to the elution volume of 2 to 2.5 *N*-acetylglucosamine oligomers (one *N*-acetylglucosamine unit is equivalent to two galactose residues in elution volume). When the trisaccharide was treated with β-galactosidase at 37°C for 18 h (Fig. 3-A-b), there was a decrease in size corresponding to one galactose residue. Treatment of the product with β-*N*-acetylhexosaminidase at 37°C for 18 h (Fig. 3-A-c) released one *N*-acetylglucosamine residue, with the remainder eluting at the position of galactitol. As a result, the carbohydrate sequence of oligosaccharide A was shown to be Gal-GlcNAc-Galol. The linkage between galactose and *N*-acetylglucosamine was deduced to be β(1-4), judging from the structures of erythrocyte poly-*N*-acetyllactosamine-type oligosaccharides [14, 15]. Renkonen *et al.* showed that the GlcNAcβ1-6Gal sequence interacted with WGA-agarose [27] so the linkage between *N*-acetylglucosamine and galactose of oligosaccharide A must be β(1-6), because oligosaccharide A was originally obtained from the WGA-bound fraction, as shown in Fig. 1. For a more convincing result, methylation analysis was performed and 1,2,3,4,5-penta-*O*-methylgalactitol was detected (data not shown). These data established the structure of oligosaccharide A to be Galβ1-4GlcNAcβ1-6Galol, as shown in Table 1.

We then tested oligosaccharide A on poly-*N*-acetyllactosamine-binding lectin-Sepharose columns (Fig. 4-A). The oligosaccharide bound to the WGA-Sepharose column and was eluted with PBS containing 0.2 M *N*-acetylglucosamine. It bound to the PWM-Sepharose column so tightly that it could not be eluted with PBS containing 0.2 M *N*-acetylglucosamine, but was eluted with 0.1 N NaOH. The trisaccharide also showed affinity for the DSA,

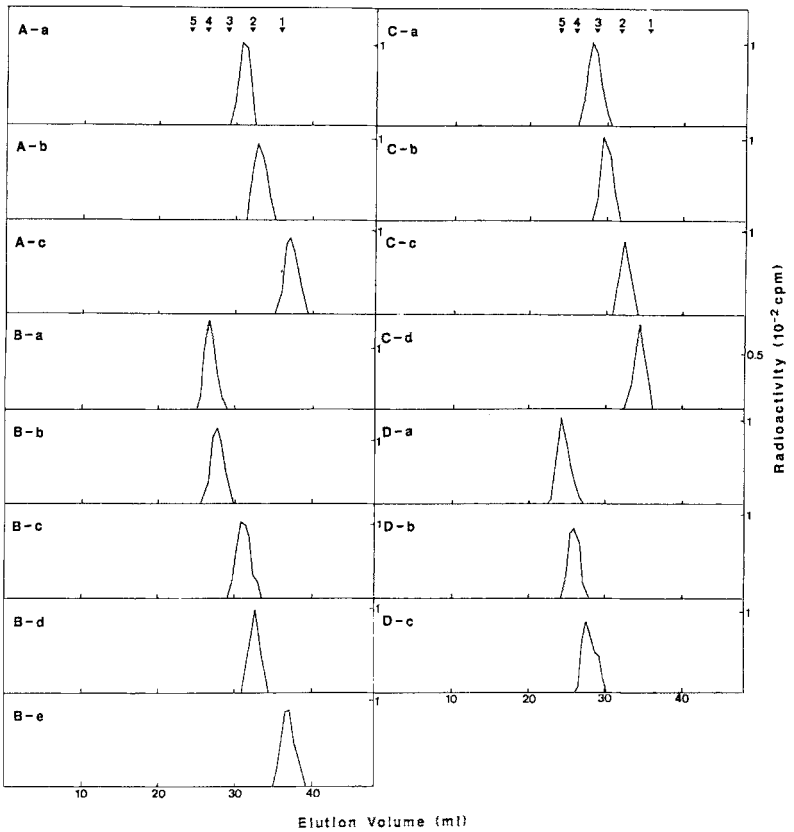

Figure 3. Elution profiles of oligosaccharides after glycosidase digestion on Bio-Gel P-4. The experimental details are given in the text. Closed triangles indicate the elution volumes of standard *N*-acetyllactosamine oligomers. (A-a); oligosaccharide A: (A-b); product of β-galactosidase treatment of (A-a): (A-c); product of β-*N*-acetylhexosaminidase treatment of (A-b). (B-a); oligosaccharide D: (B-b); product of β-galactosidase treatment of (B-a): (B-c); product of β-*N*-acetylhexosaminidase treatment of (B-b): (B-d); product of β-galactosidase treatment of (B-c): (B-e); product of β-*N*-acetylhexosaminidase treatment of (B-d). (C-a); oligosaccharide F: (C-b); product of β-galactosidase treatment of (C-a): (C-c); product of β-*N*-acetylhexosaminidase treatment of (C-b): (C-d); product of β-galactosidase treatment of (C-c). (D-a); oligosaccharide G: (D-b); product of β-galactosidase treatment of (D-a): (D-c); product of β-*N*-acetylhexosaminidase treatment of (D-b).

LEA- and STA-Sepharose columns, being retarded on the columns. From these findings, it was concluded that the five lectins tested here showed affinity to a part of the I-antigenic structure having a GlcNAcβ1-6Galol linkage like oligosaccharide A, and the affinities of WGA and PWM were stronger than those of DSA, LEA and STA.

Oligosaccharide B

We have already shown that oligosaccharide B (V-3 in Fig. 1). which bound firmly to an *Erythrina variegata* lectin (EVA)-Sepharose column, was Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galol [37]. So oligosaccharide B was used as the second standard sample containing a part of the I-antigen structure.

Fig. 4-B shows the elution profile of oligosaccharide B. As for WGA, the affinity became weaker with the substitution at the C-3 position of the galactose residue, compared to the result for trisaccharide Galβ1-4GlcNAcβ1-6Galol (oligosaccharide A). Oligosaccharide B showed high affinity for PWM and bound to the lectin-Sepharose column. Compared to oligosaccharide A, the affinity was a little weaker, because oligosaccharide B could be eluted with 0.2 M *N*-acetylglucosamine and oligosaccharide A with 0.1 M NaOH. These findings suggest that the substitution at the C-3 position of the terminal galactitol residue interferes with the interaction of oligosaccharide B with the lectin-Sepharose column. As for DSA, LEA and STA, oligosaccharide B showed weak affinity, similar to oligosaccharide A, and was retarded on the lectin-Sepharose columns.

Oligosaccharide C

We treated oligosaccharide B with β-galactosidase. The product, designated as oligosaccharide C, was GlcNAcβ1-3(GlcNAcβ1-6)Galol, as shown in Table 1. Oligosaccharide C showed weak affinity for the WGA-Sepharose column (Fig. 4-C), and no affinity for the other four lectin-Sepharose columns, suggesting that the *N*-acetyllactosamine sequence might be required for the interaction with the lectins other than WGA.

Oligosaccharides D and E

Oligosaccharide D (Fig. 3-B-a) showed a decrease in size corresponding to one galactose residue on β-galactosidase digestion (Fig. 3-B-b). The product was treated with β-*N*-acetylhexosaminidase and one *N*-acetylglucosamine residue was released (Fig. 3-B-c). Then on treatment with β-galactosidase, one galactose residue was released (Fig. 3-B-d). Finally, the product was treated with β-*N*-acetylhexosaminidase and one *N*-acetylglucosamine residue was released (Fig. 3-B-e). As a result, the carbohydrate sequence of oligosaccharide D was determined to be Gal-GlcNAc-Gal-GlcNAc-Galol. To determine the carbohydrate linkage between *N*-acetylglucosamine and galactose, methylation analysis was carried out. 3,6-Di-*O*-methylglucosaminitol, 2,4,6-tri-*O*-methylgalactitol and 1,2,3,4,5-penta-*O*-methylgalactitol were detected (data not shown). These results established the structure of oligosaccharide D to be Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-6Galol.

Oligosaccharide D, was then treated with β-galactosidase and the product, tetrasaccharide GlcNAcβ1-3Galβ1-4GlcNAcβ1-6Galol, designated as oligosaccharide E, was used as a standard sample.

As shown in Fig. 4-D and E, oligosaccharide D showed strong affinity to DSA and LEA, while oligosaccharide E showed weaker affinity. It was concluded that more than two intact *N*-acetyllactosamine repeating units were important for the affinity of DSA and LEA to

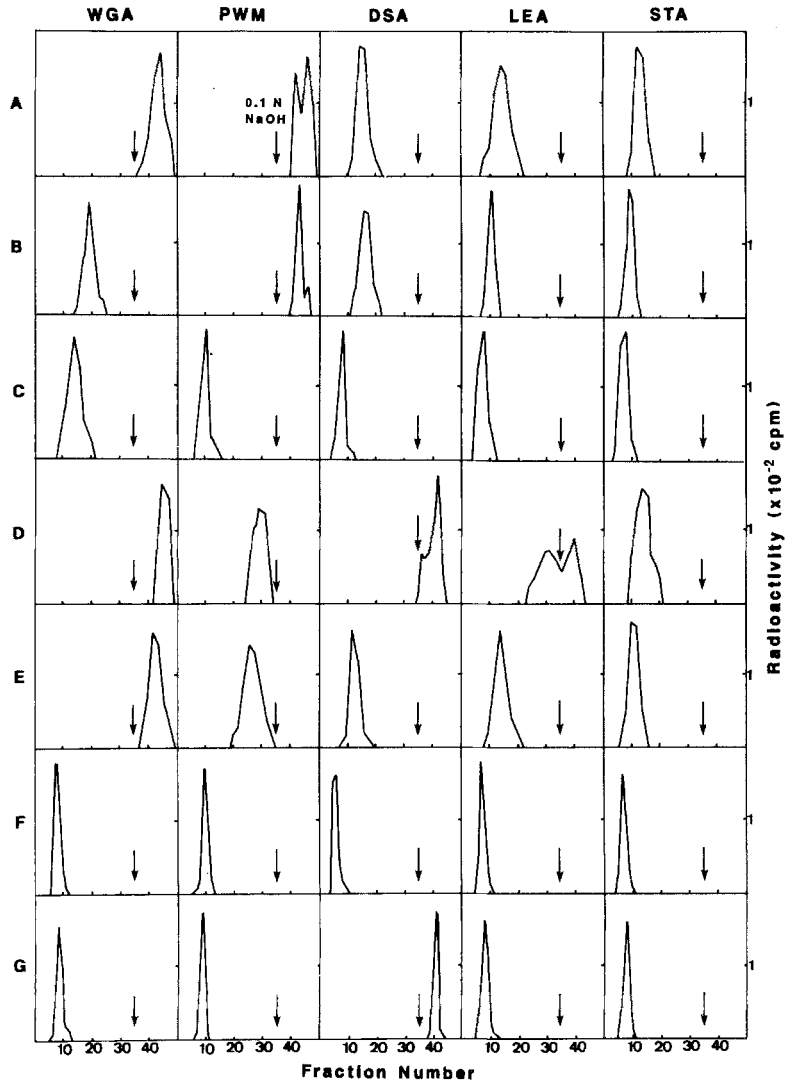

Figure 4. Lectin-affinity chromatography of the oligosaccharide fragments on poly-*N*-acetyllactosamine-binding lectin-Sepharose columns. Elution was performed with PBS and the arrows indicate the point where the buffer was changed to PBS containing 0.2 M *N*-acetylglucosamine except otherwise described. Panels A-G are the elution profiles of oligosaccharides A-G listed in Table 1. Oligosaccharides which did not bind to the column were eluted at estimated fraction number 8. WGA, wheat germ agglutinin; PWM, pokeweed mitogen; DSA, *Datura stramonium* agglutinin; LEA, *Lycopersicon esculentum* (tomato) agglutinin; STA, *Solanum tuberosum* (potato) agglutinin.

oligosaccharides. For WGA and PWM, *N*-acetyllactosamine repeating units were not important for their affinity. STA showed only weak affinity to oligosaccharides D and E, suggesting that STA might recognize a wider range of carbohydrate sequences.

Oligosaccharide F

When oligosaccharide F (Fig. 3-C-a) was incubated with β-galactosidase, there was a decrease in size corresponding to one galactose residue (Fig. 3-C-b). The product was then treated with β-*N*-acetylhexosaminidase and was converted to Gal-GlcNAcol (Fig. 3-C-c). The product was then converted to GlcNAcol by treatment with β-galactosidase (Fig. 3-C-d). To determine the linkage positions, methylation analysis was performed. 2,3,4,6-Tetra-*O*-methylgalactitol, 2,4,6-tri-*O*-methylgalactitol, 1,3,5,6-tetra-*O*-methylglucosaminitol and 3,6-di-*O*-methylglucosaminitol were detected in the molar ratio of 0.9:1.4:1.0:1.2. These results indicate that oligosaccharide F is Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcol. As shown in Fig. 4-F, oligosaccharide F did not show affinity to any of the lectin columns.

Oligosaccharide G

Oligosaccharide G was found to be a hexasaccharide consisting of three *N*-acetyllactosamine and three galactose residues (Fig. 3-D-a). Treatment with β-galactosidase released one galactose residue (Fig. 3-D-b). The product was then treated with β-*N*-acetylhexosaminidase and one *N*-acetylglucosamine residue was released (Fig. 3-D-c), suggesting that the hexasaccharide has a linear chain. From these data, the carbohydrate sequence of the hexasaccharide was shown to be Gal-GlcNAc-Gal-GlcNAc-Gal-GlcNAcol. Since the hexasaccharide did not show any affinity to the WGA-Sepharose column, it might not contain the GlcNAcβ1-6Gal structure observed for oligosaccharides A-E. Thus the structure of oligosaccharide G might be Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcol.

As shown in Fig. 4-G, oligosaccharide G bound only to the DSA-Sepharose column. Thus, as for WGA, PWM, LEA and STA, the *N*-acetyllactosamine repeating sequence is not sufficient for interaction with carbohydrate moieties, but the presence of a GlcNAcβ(1-6) linkage is required.

These results have shown that WGA, PWM, DSA, LEA and STA can interact with a part of the I-antigenic structure, and DSA may preferentially interact with the i-antigenic structure, which contains unsubstituted *N*-acetyllactosamine repeating units.

Discussion

The results of this study demonstrate that there are several kinds of poly-*N*-acetyllactosamine-binding lectins which are different in their sugar binding specificities. Galβ1-4GlcNAcβ1-6Galol strongly binds to the WGA-Sepharose column and is eluted with PBS containing 0.2 M *N*-acetylglucosamine. Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galol showed moderate affinity and was retarded on the column, suggesting that the affinity was reduced on substitution at the C-3 position of the galactitol, compared to trisaccharide Galβ1-4GlcNAcβ1-6Galol. GalNAcβ1-3(GlcNAcβ1-6)Galol also showed affinity to the column, suggesting that the minimum structure which is important for the interaction with WGA-Sepharose is the GlcNAcβ1-6Gal sequence found by Renkonen *et al.* [27].

Table 1. Carbohydrate-binding specificities of several poly-*N*-acetyllactosamine-binding lectins.

Oligosaccharide		WGA	PWM	DSA	LEA	STA
A	Galβ1-4GlcNAcβ1-6Galol	S[a]	S	W	W	W
B	Galβ1-4GlcNAcβ1 6 Galol 3 Galβ1-4GlcNAcβ1	M[b]	S	W	W	W
C	GlcNAcβ1 6 Galol 3 GlcNAcβ1	W[c]	-	-	-	-
D	Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-6Galol	S	M	S	S	W
E	GlcNAcβ1-3Galβ1-4GlcNAcβ1-6Galol	S	M	W	W	W
F	Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcol	-[d]	-	-	-	-
G	Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcol	-	-	S	-	-

[a] S, strong binding (eluted at estimated fraction number >35).
[b] M, moderate binding (eluted at estimated fraction number 20-35).
[c] W, weak binding (eluted at estimated fraction number 8-20).
[d] -, no binding at all (eluted at estimated fraction number 8).

Tetrasaccharide Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcol and hexasaccharide Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcol, which contain the i-antigenic structure, did not show any affinity for the WGA-Sepharose column at all.

As for PWM, Galβ1-4GlcNAcβ1-6Galol bound to the column so tightly that it could not be eluted with the buffer containing *N*-acetylglucosamine, but was eluted with 0.1 N NaOH. Pentasaccharide Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galol also showed strong affinity for the PWM-Sepharose column, but was eluted with *N*-acetylglucosamine, suggesting that on substitution at the C-3 position of the galactitol residue the affinity became a little lower. GlcNAcβ1-3(GlcNAcβ1-6)Galol did not show any affinity at all, suggesting that the *N*-acetyllactosamine (Galβ1-4GlcNAc) sequence is indispensable. However, it is not enough for interaction with the PWM-Sepharose column, because tetrasaccharide Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcol and hexasaccharide Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcol did not show any affinity for the PWM-Sepharose column. From these findings, the minimum structural requirement for interaction with the PWM-Sepharose column was concluded to be Galβ1-4GlcNAcβ1-6Galol.

As for DSA, parts of the I-antigenic structure, Galβ1-4GlcNAcβ1-6Galol and Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galol were retarded on the columns. Compared to WGA and PWM, the affinity to the I-antigenic structure was weaker. GlcNAcβ1-3(GlcNAcβ1-6)-Galol did not interact with the DSA-Sepharose column at all, suggesting that the *N*-acetyllactosamine structure is indispensable. Although tetrasaccharide Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcol did not interact with the column, hexasaccharide Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcol bound to the column and was eluted with 0.2 M *N*-acetylglucosamine. Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-6Galol also bound to the DSA-Sepharose column, conversely GlcNAcβ1-3Galβ1-4GlcNAcβ1-6Galol showed very weak affinity. It is concluded that more than two intact *N*-acetyllactosamine repeating units may be essential for the binding to the DSA-Sepharose column. This result is in good agreement with the results of Cummings and Kornfeld [30] and Yamashita *et al.* [31] who showed that complex-type carbohydrate moieties containing *N*-acetyllactosamine repeating units bind to a DSA-Sepharose column. These groups reported that complex-type tri-antennary and tetra-antennary carbohydrates containing mannose residues substituted at the C-2,6 positions by *N*-acetyllactosamine also interact with a DSA-Sepharose column. Taking into account these data, two *N*-acetyllactosamine units may interact with a DSA-Sepharose column.

As for LEA and STA, Galβ1-4GlcNAcβ1-6Galol and Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galol were retarded on the columns. Compared to WGA and PWM, LEA and STA showed weaker affinity to these oligosaccharides which contain a part of the I-antigenic structure. LEA strongly interacted with a rather wide range of oligosaccharide sequences containing the i and I-antigenic structures, like pentasaccharide Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-6Galol. The product of β-galactosidase treatment of this pentasaccharide did not show strong affinity, suggesting that the *N*-acetyllactosamine repeating sequence was important. The oligosaccharide fragments listed in Table 1 did not show strong affinity to STA. It was reported that the inhibitory effect of N-peracetylated chitin oligosaccharides on STA increased with increased chain length up to the tetraose [37] suggesting that STA has an extended binding site. STA may recognize a wider range of oligosaccharide sequences than LEA or other poly-*N*-acetyllactosamine binding lectins.

In the present study, we determined the carbohydrate binding specificities of several poly-*N*-acetyllactosamine-binding lectins, as summarized in Table 1. These lectins may be useful for biochemical and histological analyses.

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