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Structural Requirements for the Binding of Oligosaccharides to Immobilized Lectin of *Erythrina variegata (Linn)* var. *Orientalis*

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The structural requirements for the interaction of asparagine-linked oligosaccharide moieties of glycoproteins with *Erythrina variegata* agglutinin (EVA) were investigated by means of affinity chromatography on an EVA-Sepharose column. Some of the branched poly-*N*-acetyllactosamine-type oligosaccharides obtained from human erythrocyte band 3 glycoprotein were found to show high affinity to EVA-Sepharose, whereas complex-type oligosaccharides were shown to have low affinity. Hybrid type, oligomannose-type and unbranched poly-*N*-acetyllactosamine-type oligosaccharides bound very little or not at all to EVA-Sepharose. To further study the carbohydrate-binding specificity of this lectin, we investigated the interaction of immobilized EVA and oligosaccharide fragments obtained through partial hydrolysis from branched poly-*N*-acetyllactosamine-type oligosaccharides. Branched poly-*N*-acetyllactosamine-type oligosaccharides were subjected to limited hydrolysis with 0.1% trifluoroacetic acid at 100°C for 40 min and then separated on an amino-bonded silica column. One of pentasaccharides thus prepared strongly bound to the EVA-Sepharose column. Structural analysis of this pentasaccharide showed that the Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Gal sugar sequence, which is an I-antigen determinant, was essential for the high affinity binding of the oligosaccharides to the EVA-Sepharose column.

Lectins, which are cell-agglutinating and carbohydrate-binding proteins, occur in many plants, particularly legumes [1], and also in bacteria, fungi and animals [2]. Owing to the strict specificity of their binding with oligosaccharides, they have been widely applied in cell biology, biochemical separation, and other fields. A large number of lectins of plant and other origins have been identified and purified to homogeneity, and studied with regard to their physicochemical properties and carbohydrate specificities. Trees and shrubs of the

Abbreviations: EVA, *Erythrina variegata* agglutinin; WGA, wheat germ agglutinin; STA, potato lectin; LEA, tomato lectin; DSA, *Datura stramonium* agglutinin; PBS, 0.01 M sodium phosphate buffer, pH 7.3, containing 0.15 M NaCl; Galol, galactitol.

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genus *Erythrina* are widely distributed throughout the tropics and subtropics. A number of *Erythrina* lectins have been purified and characterized to varying degrees (*E. arborescens* [3], *E. caffra* [4], *E. corallodendron* [4-6], *E. cristagalli* [4, 6], *E. edulis* [7], *E. flabelliformis* [4], *E. humeana* [4], *E. indica* [3, 8], *E. latissima* [4, 6], *E. litosperma* [3], *E. lysistemon* [4, 6], *E. perrieri* [4], *E. stricta* [4], *E. suberosa* [3], *E. variegata* [9, 10], *E. zeyheri* [4]). All *Erythrina* lectins studied demonstrated the striking similarities in molecular properties and simple sugar specificities and the hemagglutinating activity of these lectins is specifically inhibited by galactose, lactose, *N*-acetylgalactosamine and *N*-acetyllactosamine. However, the essential sugar sequence for the strong binding to these lectins has not been elucidated in detail. A lectin of deigo (*Erythrina variegata* var. *Orientalis*) seeds was first reported in 1984 by Fukuda *et al.* [9]. A hemagglutination inhibition test involving various simple sugars, using human O type erythrocytes, indicated that the hemagglutinating activity of the lectin was inhibited by galactose.

Moreover, the lectins of the *Erythrina* species were also found to exhibit leukocyte agglutinating activity. Thus, *Erythrina variegata* agglutinin (EVA) may be an important tool for studying glycoproteins on the cell surface. This paper describes the structural requirements for the binding of oligosaccharides to immobilized EVA.

Materials and Methods

Materials

The deigo (*Erythrina variegata var orientalis*) seeds used were a generous gift from Dr. Nobuhiro Fukuda (University of the Ryukyu, Nishinara, Okinawa, Japan). Pronase was from Kaken Chemical Co. (Tokyo, Japan). Endo-β-galactosidase, β-*N*-acetylhexosaminidase and β-galactosidase were purchased from Seikagaku Kogyo Co., Ltd. (Tokyo, Japan). Sephadex G-25 and G-50, DEAE-Sephacel, and molecular mass markers for gel filtration and SDS-polyacrylamide gel electrophoresis were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Dowex 50W-X8 and Bio-Gel P-4 (-400 mesh) were purchased from Bio-Rad (Richmond, CA, USA). NaB^3H_4 (74 GBq/mmol) was from New England Nuclear (Boston, MA, USA). The standard samples of complex-type, oligomannose-type, hybrid-type, branched poly-*N*-acetyllactosamine-type and unbranched poly-*N*-acetyllactosamine-type oligosaccharides were prepared in this laboratory [11-14].

Purification of Erythrina variegata Agglutinin (EVA)

All procedures were carried out at 4°C. Approximately 120 g of deigo seeds were homogenized with a blender in 1 l of 0.01 M sodium phosphate buffer, pH 7.3, containing 0.15 M NaCl (PBS). The mixture was allowed to stand overnight under constant stirring and then centrifuged at 8,500 x *g* for 30 min. Solid ammonium sulfate was added to the supernatant to 80% saturation, and the resultant precipitate was dissolved in distilled water, and dialysed first against distilled water and then PBS. After the lectin solution had been centrifuged at 8,500 x *g* for 1 h to remove insoluble materials, the clear supernatant was applied to a column (1.5 x 25 cm) of lactose-Sepharose, which had been equilibrated with PBS. The column was washed with PBS until the absorbance at 280 nm became approxi-

mately 0.01 and EVA was eluted with PBS containing 0.2 M lactose. Agglutination activity was determined using human O type erythrocytes. About 100 mg of EVA was obtained from 120 g of deigo seeds. The purified EVA was subjected to protein sequence analysis by Edman degradation using a MilliGen ProSequencer 6600 solid phase sequenator.

SDS-Polyacrylamide Gel Electrophoresis

SDS-Polyacrylamide gel electrophoresis was performed according to the method of Laemmli [15] with 13% acrylamide gel. The samples were heated at 100°C for 3 min in the presence of 2% SDS prior to electrophoresis. For determination of the apparent molecular mass of the subunits, the following markers were used: phosphorylase b (M_r 94,000), bovine serum albumin (67,000], ovalbumin (43,000) and carbonate dehydratase (30,000). The gel was stained with 0.2% Coomassie Brilliant Blue R-250 and destained with 10% acetic acid.

Conjugation of EVA to Sepharose

CNBr-Activated Sepharose 4B (10 ml) was washed sequentially with cold distilled water, and suspended in PBS containing EVA and left for 16 h and 4°C. Lactose (0.2 M), was added to protect the binding site of the lectin. Then the Sepharose was sequentially washed with distilled water and 0.01 M sodium phosphate buffer, pH 7.3.

Preparation of Oligosaccharides

Glycoproteins from human B and O type erythrocytes were obtained as described previously [16] and incubated with Pronase in 0.05 M sodium borate buffer, pH 7.8, containing 1 mM $CaCl_2$. Incubation was carried out for 72 h at 37°C. After the reaction mixture had been centrifuged at 15,000 x *g* for 1 h, the supernatant was applied to a column (1.5 x 100 cm) of Sephadex G-25, which had been equilibrated with distilled water, followed by elution with distilled water. Glycopeptides were monitored by the phenol/H_2SO_4 method [17]. The glycopeptides obtained were subjected to hydrazinolysis to release asparagine-linked sugar chains as described previously [18]. The oligosaccharides released were dissolved in 2 ml of a 4.5 M sodium acetate solution and then N-acetylated at room temperature by the addition of 0.1 ml of acetic anhydride, five times at 10 min intervals. Then the reaction mixture was applied to a column (1.5 x 120 cm) of Sephadex G-25, followed by elution with distilled water at room temperature. Neutral sugars were assayed by the phenol/H_2SO_4 method [17]. The fractions containing oligosaccharides were directly applied to a DEAE-Sephacel column (1.3 x 10 cm), which had been equilibrated with 2 mM Tris/HCl, pH 7.4. After washing the column with about 30 ml of the same buffer, elution was performed with a linear concentration gradient of NaCl, from 0 to 0.2 M, in 140 ml of 2 mM Tris/HCl buffer (pH 7.4). The neutral oligosaccharides were eluted first from the column. They were further fractionated on a column of Bio-Gel P-4 as described previously [19].

Radioisotope Labeling of Oligosaccharides

Tritiated borohydride reduction was performed according to the method of Takasaki and Kobata [20]. About 100 nmol of oligosaccharides was reduced with 5 µmol (9.25 MBq) of NaB^3H_4 in 50 µl of 0.01 N NaOH at 25°C for 16 h. Ten mg of $NaBH_4$ was then added, and

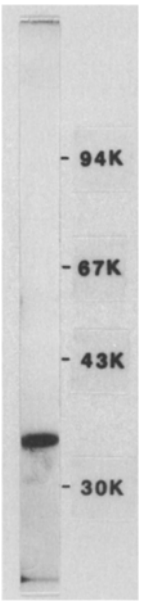

Figure 1. SDS-polyacrylamide gel electrophoresis of the purified EVA lectin on a lactose-Sepharose column. The experimental details are given in the Materials and Methods section. Numbers indicate the migration positions of the molecular mass standards.

the reaction was allowed to continue at 25°C for another 2 h. The reaction was stopped by adding one drop of glacial acetic acid. After the mixture had been passed through a small column of Dowex 50W-X8 (H^+ form; 0.5 x 5.0 cm), the boric acid was removed by repeated evaporation with methanol. Radioactive contaminants due to NaB^3H_4 were removed by descending paper chromatography for 18 h on Whatman 3MM paper with ethyl acetate/acetic acid/formic acid/H_2O, 18/3/1/4 by vol. Radioactivity was monitored with a radiochromatogram scanner (Packard, model 7220; Packard Instrument Co., Inc., Gowners Grove, IL, USA). The radioactive peak which remained at the origin was eluted with water.

Affinity Chromatography of the Labeled Oligosaccharides on an EVA- Sepharose Column

A radioactively labeled sample (10^3 cpm) in a total volume of 50 µl was applied to an EVA-Sepharose column (0.6 x 7.0 cm), equilibrated with PBS and allowed to stand at room temperature for 1 h. Elution was performed with the same buffer and then with 0.2 M lactose at the flow rate of 2 ml/h, fractions of 0.5 ml being collected. The recovery of the radioactivity in all experiments was more than 95%

Composition and Methylation Analyses

The carbohydrate compositions of the EVA-bound and EVA-unbound fractions were determined by gas chromatography on a capillary column (0.2 mm x 25 m) of CBP-5 (Shimadzu, Kyoto, Japan). The carbohydrates were chromatographed as alditol acetates after hydrolysis with 2 M HCl at 100°C for 2 h. The EVA-bound and EVA-unbound fractions were methylated by the method of Ciucanu and Kerek [21].

After hydrolysis, reduction and acetylation, the alditol acetates of partially methylated sugars were analyzed with a gas chromatograph-mass spectrometer according to the previously described method [12, 13]. Each peak was identified by gas chromatograph-mass spectrometry, GCMS-QP 1000 (Shimadzu), on a column (0.2 mm x 25 m) of CBP-5. The conditions for the mass spectrometry were as follows: ion source temperature, 250°C; ionizing potential, 70 ev; trap current, 60 μA.

Preparation of EVA-Bound and EVA-Unbound Fragments, and Determination of Their Sugar Sequences

The branched poly-*N*-acetyllactosamine-type oligosaccharides obtained by Bio-Gel P-4 column chromatography were subjected to limited hydrolysis with 0.1% trifluoroacetic acid at 100°C for 40 min, according to the method of Renkonen *et al.* [22], and then radioisotope-labeled and reduced with NaB^3H_4. The hydrolysis mixture was fractionated by normal phase HPLC on a Lichrosorb-NH_2 (Merck, Darmstadt, W. Germany) column, using a 50 ml linear gradient of 75-50% acetonitrile in H_2O at a flow rate of 1 ml/min [14]. The molecular size was determined by HPLC on a Bio-Gel P-4 column. The affinity to EVA of various fragments was examined by affinity chromatography.

Exoglycosidase Digestion of Oligosaccharides

Sugar sequencing was performed by the sequential exoglycosidase treatment method. Digestions with β-galactosidase and β-*N*-acetylhexosaminidase from jack beans were carried out in 50 mM sodium acetate buffer, pH 4.0. The samples were incubated at 37°C under a toluene atmosphere for 24 h. The digestion with endo-β-galactosidase from *Escherichia freundii* was carried out in acetate buffer, pH 5.8. The reaction mixture was heated at 100°C for 3 min to terminate the reaction, and then passed through a small column of Dowex 50W-X8. (H^+ form; 0.5 x 5.0 cm).

Gel Permeation Chromatography

The purity and molecular sizes of oligosaccharides were determined using a high performance liquid chromatograph equipped with two columns (0.72 x 100 cm) of Bio-Gel P-4. Elution was performed with water at the flow rate of 0.3 ml/min. During this operation, the columns were maintained at 55°C by circulating warm water in jackets. The eluate was monitored with an ultraviolet spectrophotometer. *N*-Acetylglucosamine oligomers were used as standards. Fractions of 0.45 ml were collected and aliquots were subjected to radioactivity counting with a liquid scintillation counter.

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 1                                          11
 X -Glu-Thr-Ile-Ser-Phe-Ser-Phe-Ser-Glu-Phe-Glu-Ala-Gly-Asn-Asp-Asn-Leu-Thr-Leu-

21                                          31
Gln-Gly-Ala-Ala-Leu-Ile-Thr-Gln-Ser-Gly-Val-Leu-Gln-Leu- X -Lys-Ile-Asn-Gln-Asn-
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Figure 2. Amino terminal sequence of purified EVA.

Results

Fig. 1 shows the electrophoretic behavior of the purified lectin in an SDS-polyacrylamide gel. The purified lectin gave a single band of molecular weight 33 kDa in the absence of 2-mercaptoethanol. The partial N-terminal amino acid sequence of the purified EVA was determined and the results are shown in Fig. 2. The N-terminal amino acid sequence of EVA is nearly identical with those of other *Erythrina* lectins reported by Lis *et al.* [4].

Of the various oligosaccharides tested for inhibition of hemagglutination by EVA, an oligomannose-type oligosaccharide having the sequence, Manα1-3(Manα1-6)Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc, had no effect up to a concentration of 1 mg/ml, whereas a complex-type bi-antennary oligosaccharide, Galβ1-4GlcNAcβ1-2Manα1-3(Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4(Fucα1-6)GlcNAc inhibited four hemagglutinating doses of EVA at the concentration of 32 μg/ml. Poly-*N*-acetyllactosamine-type oligosaccharide N-I of human erythrocyte band 3 glycoprotein [11], was four times more active than the above complex-type bi-antennary oligosaccharide.

We then examined the affinity of several asparagine-linked oligosaccharides for an EVA-Sepharose column. When an oligomannose-type oligosaccharide, $(Man)_5$-GlcNAcol, derived from porcine thyroglobulin, was applied to the column of EVA-Sepharose, it was recovered without retardation (Fig. 3a). The unbranched poly-*N*-acetyllactosamine-type oligosaccharides prepared from human K562 erythroblast cells were also eluted in the void volume fraction of the column (Fig. 3b). Furthermore, a hybrid-type oligosaccharide, Gal-GlcNAc-$(Man)_5$-GlcNAcol, derived from human thyroglobulin, interacted weakly with EVA (Fig. 3c).

The complex-type oligosaccharide, $(Gal)_3$-$(GlcNAc)_3$-$(Man)_3$-$(GlcNAc)_2$, was retarded on the column (Fig. 3d). A part of the branched poly-*N*-acetyllactosamine-type oligosaccharides derived from human erythrocyte band 3 glycoprotein, the structures of which were described previously [11], were absorbed to the column and eluted with lactose (Fig. 3e). The *N*-acetylglucosamine oligomers, $(GlcNAc)_2$, $(GlcNAc)_4$ or $(GlcNAc)_6$, did not bind to the EVA-Sepharose column (Fig. 3f). It is concluded that EVA shows weak affinity for complex-type glycopeptides possessing two *N*-acetyllactosamine side chains. This is in good agreement with the data reported by Lis *et al.* [4] and Debray *et al.* [6]. Hybrid-type

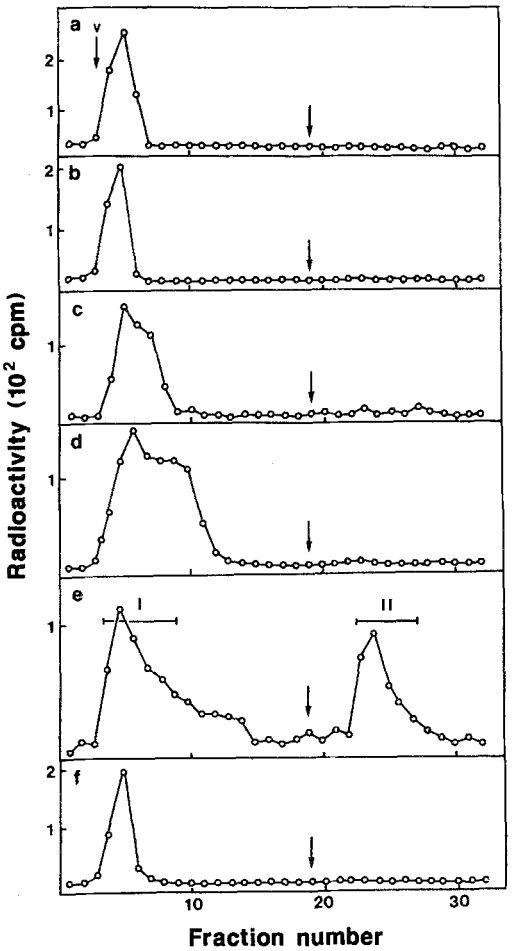


Figure 3. Affinity chromatography of oligosaccharides on an EVA-Sepharose 4B column. Elution was performed with 10 mM sodium phosphate buffer, pH 7.3, containing 0.15 M NaCl, followed by the same buffer containing 0.2 M lactose at a flow rate of 2 ml/h, with 0.5 ml fractions being collected. An arrow, V, indicates the void volume fraction and the other arrow indicates the position where the buffer was changed. a) oligomannose-type oligosaccharide, Manα1-3(Manα1-6)Manα1-6(Manα1-3)Manβ1-4GlcNAc; b) hybrid-type oligosaccharide, Manα1-3(Manα1-6)Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAc; c) complex-type oligosaccharide, Galβ1-4GlcNAcβ1-2Manα1-3(Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc; d) branched poly-*N*-acetyllactosamine-type oligosaccharides; e) unbranched poly-*N*-acetyllactosamine-type oligosaccharides; f) $(GlcNAc)_4$. These oligosaccharides were radiolabeled by the reduction with NaB^3H_4.

glycopeptides, which have one *N*-acetyllactosamine side chain, interact weakly with EVA-Sepharose. Oligomannose-type oligosaccharides, which do not possess *N*-acetyllactosamine moieties, do not interact with EVA-Sepharose at all.

Table 1. Carbohydrate composition analysis of the EVA-bound and EVA-unbound oligosaccharide fractions of branched poly-*N*-acetyllactosamine-type oligosaccharides from human erythrocyte band 3 glycoprotein.

	Molar ratio[a]			
	Fuc	Man	Gal	GlcNAc
EVA-bound (I)	2.4	3.0	14.0	18.1
EVA-unbound (II)	2.7	3.0	8.1	10.5

[a] Values are expressed in relation to mannose, which was taken as 3.0.

Carbohydrate composition analysis (Table 1) showed that the EVA-bound poly-*N*-acetyllactosamine-type oligosaccharide fraction (Fig. 3e, II) had a higher content of galactose and *N*-acetylglucosamine than the EVA unbound (including retarded) fraction (Fig. 3e, I). Methylation analysis showed that the carbohydrate chains of the EVA-bound fraction contain much more branched galactose residues (2,4-di-*O*-methyl-D-galactose) compared with the EVA-unbound fraction (Table 2). Furthermore, the EVA bound fraction was more resistant to endo-β-galactosidase digestion than the EVA-unbound fraction (data not shown), indicating that the bound fraction is more branched than the unbound fraction.

Secondly, in order to clarify the structural elements responsible for the high affinity of branched poly-*N*-acetyllactosamine-type oligosaccharides for the immobilized EVA, the poly-*N*-acetyllactosamine-type oligosaccharides derived from human erythrocyte band 3 glycoprotein were subjected to limited hydrolysis with trifluoroacetic acid and then tritium-labeled by reduction with NaB^3H_4. The mixture was then fractionated on a Lichrosorb-NH_2 column according to the method described elsewhere [14]. The affinity for EVA of various fragments was examined by affinity chromatography on an EVA-Sepharose column. Disaccharides, trisaccharides and tetrasaccharides did not bind to the EVA column. Some (25%) pentasaccharides bound to the column. The pentasaccharide fragment(s) absorbed to the EVA-Sepharose column was named EVA(+)-5, which corresponded to 3.5 *N*-acetylglucosamine units on a column of Bio-Gel P-4 (Fig. 4a), while the void column fragments, called EVA(-)-5, corresponded to 4.0 *N*-acetylglucosamine residues (Fig. 4d). After treatment with β-galactosidase, the decrease in size of EVA(+)-5 corresponded to a loss of 2.0 galactose residues (Fig. 4b), and the agalacto-EVA(+)-5 was not absorbed to the EVA-Sepharose column. When β-galactosidase-treated EVA(+)-5 was further digested with β-*N*-acetylhexosaminidase, a decrease in size corresponding to 2.0 *N*-acetylglucosamine residues was observed (Fig. 4c). The final product corresponded to 0.5 *N*-acetylglucosamine residues (namely, 1.0 galactose residue). Similarly, EVA(-)-5 was digested sequentially with β-galactosidase and β-*N*-acetylhexosaminidase twice, leaving galactosaminitol as a final product (Fig. 4d-h).

Table 2. Methylation analysis of the EVA-bound and EVA-unbound fractions of branched poly-*N*-acetyllactosamine-type oligosaccharides derived from human erythrocyte band 3 glycoprotein.

		Fraction	
Methylated sugar	Position of O-Me	EVA-bound	EVA-unbound
Fuc	2,3,4	0.4	1.3
Gal	2,3,4,6	3.5	2.6
	2,4,6	6.6	3.3
	2,4	5.7	0.7
Man	3,4,6	2.3	1.6
	2,4	0.3	1.0
	3,6	0.4	0.4
GlcNAc	3,6	14.6	8.6

Values are expressed in relation to the total amount of mannose derivatives, which was taken as 3.0.

From the above results and from the methylation analysis data in Table 3, the sugar sequences of the EVA(+)-5 and EVA(-)-5 oligosaccharides were deduced to be as shown in Fig. 5. This indicates that the pentasaccharide, Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Gal, is essential for the high affinity binding to an EVA-Sepharose column.

Discussion

Debray *et al.* reported that sugar binding specificity of four immobilized *Erythrina* lectins was directed toward unmasked *N*-acetyllactosamine sequences [6]. In the present study, we found that EVA showed high affinity for some of poly-*N*-acetyllactosamine-type oligosaccharides derived from human erythrocyte band 3 glycoprotein. On the basis of composition and methylation analyses of EVA-bound and EVA-unbound poly-*N*-acetyllactosamine-type oligosaccharides, it was found that branched galactose residues were abundant in the EVA-bound fraction compared to the EVA-unbound fraction. This indicates that branched galactose residues may participate in the high affinity binding to an EVA-Sepharose column. To identify the minimal essential structure for EVA binding, we prepared partial hydrolysis fragments from branched poly-*N*-acetyllactosamine-type sugar chains. Disaccharides, trisaccharides and tetrasaccharides did not bind to EVA-Sepharose. In contrast, among the pentasaccharides, Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galol strongly bound to the column. After the removal of its non-reducing terminal galactose residues, this oligosaccharide was unable to interact with EVA. Complex-type oligosaccharides with two Galβ1-4GlcNAcβ1- branches did not firmly bind to EVA-Sepharose. These results indicate that two non-reducing terminal β-galactose residues located at appropriate positions or distance are important for the high affinity binding to EVA. Such high affinity binding to EVA as that of Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galol may be ascribed to the interaction of the two β-galactose residues of this pentasaccharide with sugar-binding site(s) of EVA.

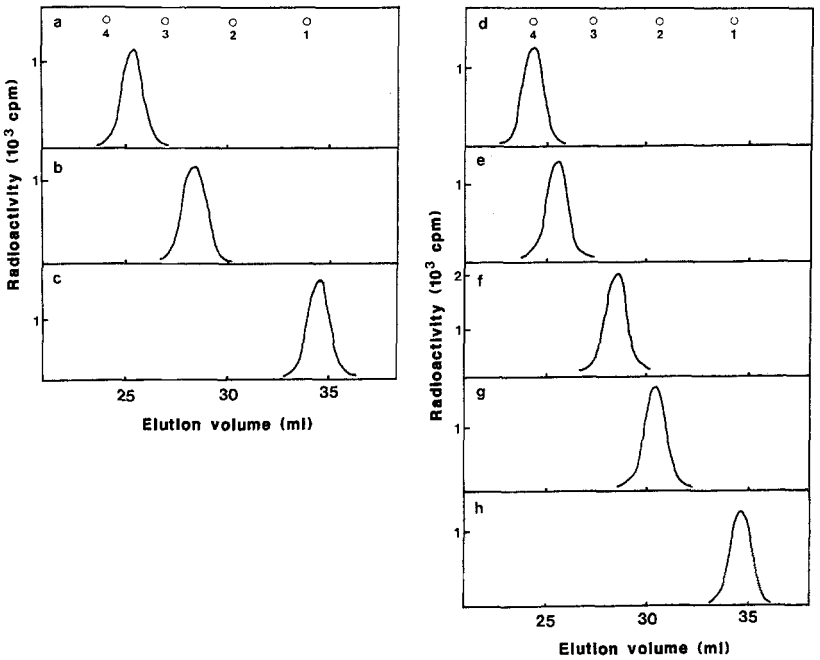
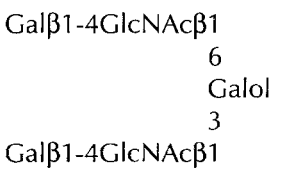


Figure 4. Elution profiles of oligosaccharides EVA(+)-5 and EVA(-)-5 after glycosidase digestion on a Bio-Gel P-4 column. The NaB^3H_4-reduced oligosaccharides were analyzed by high performance gel filtration chromatography. Open circles (1-4) indicate the elution positions of standard *N*-acetylglucosamine oligomers. a) Oligosaccharide EVA(+)-5; b) product of β-galactosidase treatment of a); c) product of β-*N*-acetylhexosaminidase treatment of b); d) original oligosaccharide EVA(-)-5; e) product of β-galactosidase treatment of d); f) product of β-*N*-acetylhexosaminidase treatment of e); g) product of β-galactosidases treatment of f); h) product of β-*N*-acetylhexosaminidase treatment of g).

EVA(+)-5.



EVA(-)-5. Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galol

Figure 5. Proposed structures for oligosaccharides EVA(+)-5 and EVA(-)5.

Table 3. Methylation analysis of oligosaccharides EVA(+)-5 and EVA(-)-5.

Methylated sugar	Position of O-Me	Oligosaccharide[a] EVA(+)-5	EVA(-)-5
Gal	2,3,4,6	2.0	1.0
	2,4,6	0.0	1.3
	2,4	0.7	0.0
GlcNAc	3,6	2.6	2.7

[a] Values are expressed in relation to 2,3,4,6 tetra *O*-methylgalactitol of EVA(+)-5 or EVA(-)-5, which was taken as 2.0 or 1.0, respectively.

In the present study, we found that some of poly-*N*-acetyllactosamine-type oligosaccharides interact with EVA strongly. Several lectins, wheat germ agglutinin (WGA) [23], pokeweed mitogen [24], potato lectin (STA) [14], tomato lectin (LEA) [25] and *Datura stramonium* agglutinin (DSA) [26], have been reported to interact with poly-*N*-acetyllactosamine-type oligosaccharides. All of these lectins are inhibited by *N*-acetylglucosamine and its (1-4)-linked oligomers, although EVA is not. This difference in sugar-binding specificity between EVA and these lectins was confirmed by using two pentasaccharides, Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galol and Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-6Galol. The former firmly binds to EVA but the interaction with the GlcNAc-binding lectins (WGA, STA, LEA and DSA) is weak [14]. On the other hand, the latter strongly binds to WGA, LEA and DSA, but not to EVA [14]. This suggests that WGA, LEA, STA and DSA preferentially bind to a GlcNAcβ(1-6)- moiety, whereas EVA binds to *N*-acetyllactosamine residues of the branched poly-*N*-acetyllactosamine-type sugar chain.

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