J. Biochem. 123, 715-721 (1998)

Synthesis of Artificial Glycoconjugate Polymers Starting from Enzymatically Synthesized Oligosaccharides and Their Interactions with Lectins[1]

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Received for publication, November 4, 1997

Styrene derivatives substituted with *N*-linked β-anomeric oligosaccharides were synthesized *via* a simple two-step procedure starting from three enzymatically prepared oligosaccharides: *N*-acetyllactosamine (Galβ1-4GlcNAc), *N*-acetylisolactosamine (Galβ1-6GlcNAc), and 4′-galactosyllactose (Galβ1-4Galβ1-4Glc). Their homo- and copolymerization with acrylamide using 2,2′-azobisisobutyronitrile as an initiator in dimethyl sulfoxide at 60°C gave the corresponding glycopolymers. Binding between glycopolymers and lectins was investigated by means of hemagglutination inhibition experiments. The inhibition of RCA_{120} lectin-induced hemagglutination by *N*-acetyllactosamine-carrying homopolymer was about 10^3 times stronger than that of the oligosaccharide itself. The enhanced binding capacity with lectins can be explained in terms of a multivalent or cluster effect along the polymeric chain. In some combinations between lectins and polymers, the copolymers inhibited hemagglutination more strongly than the homopolymers did. *N*-Acetyllactosamine-carrying glycopolymer showed about 3×10^3 times weaker inhibition of DSA lectin-induced hemagglutination than the different type of *N*-acetyllactosamine-carrying glycopolymer which has an *O*-linked β-anomeric phenyl aglycon of each repeating unit along a polyacrylamide backbone.

Key words: biomaterials, glycopolymers, lectins, oligosaccharides.

Water-soluble synthetic polymers carrying various kinds of pendant mono- and oligosaccharide components are an important class of bioactive macromolecules (*1-3*). These glycopolymers have found a variety of biomedical applications such as culture substrata (*4*), tumor diagnosis (*5-7*), human vaccines, detection and trapping of viruses (*8-10*) and toxins (*11*), and targeted drug delivery systems. These functions are attributable to biological recognition phenomena (*12-17*) induced by lectins and anti-carbohydrate monoclonal antibodies.

We reported a simple synthetic method of styrene derivatives substituted with *N*-linked glycosides *via* the following two-step procedure (*18*). (i) Introduction of an amino function at the reducing end of an oligosaccharide (*19*) and (ii) amidation with *p*-vinylbenzoyl chloride. As shown in Scheme 1, we have now applied this simple method to three kinds of biologically important oligosaccharides (*20, 21*), that is, *N*-acetyllactosamine (Galβ1-4GlcNAc) (**1a**), *N*-acetylisolactosamine (Galβ1-6GlcNAc) (**1b**), and 4′-galactosyllactose (Galβ1-4Galβ1-4Glc) (**1c**). These galactosyl oligosaccharides were prepared *via* transglycosidation using β-galactosidases of different origins. Scheme 1 also illustrates the chemical structures and their abbreviations of the monomeric styrene derivatives (**2a**, **2b**, and **2c**) and their homopolymers (**3a**, **3b**, and **3c**).

Our strategy for the molecular design of glycopolymers is to construct amphiphilic structures by arranging hydrophobic polystyrene main chains and hydrophilic pendant oligosaccharides (*4*). We reported that amphiphilic glycopolymers of this type show unique structural, physical, and biological characteristics. The latter part of this paper deals with the interactions of these glycopolymers with various lectins by means of assay of inhibition of hemagglutinating activity and two-dimensional immuno diffusion in agar.

EXPERIMENTAL PROCEDURES

General—NMR spectra were recorded with a JEOL JNM-FX-270 Fourier transform NMR spectrometer. IR spectra were taken with a Japan Spectroscopic (JASCO) A-3 grating infrared spectrophotometer. Optical rotations were determined with a JASCO DIP-181 digital polarimeter using a water-jacketed 1 dm cell at 25°C. Size exclusion chromatography (SEC) was conducted with a JASCO BIP-1 high-performance liquid chromatograph using Shodex KF-803+KF-804 columns and dimethyl sulfoxide (Me_2SO) as the eluent, and with a JASCO 800 high-performance liquid chromatograph on Shodex B804+B805 columns for copolymers using water as the eluent. Thin layer chromatography (TLC) was carried out with

[1] Funding for the Regional Leading Research Project of Shizuoka Prefecture from the Ministry of Science and Technology of Japan, is greatly appreciated.

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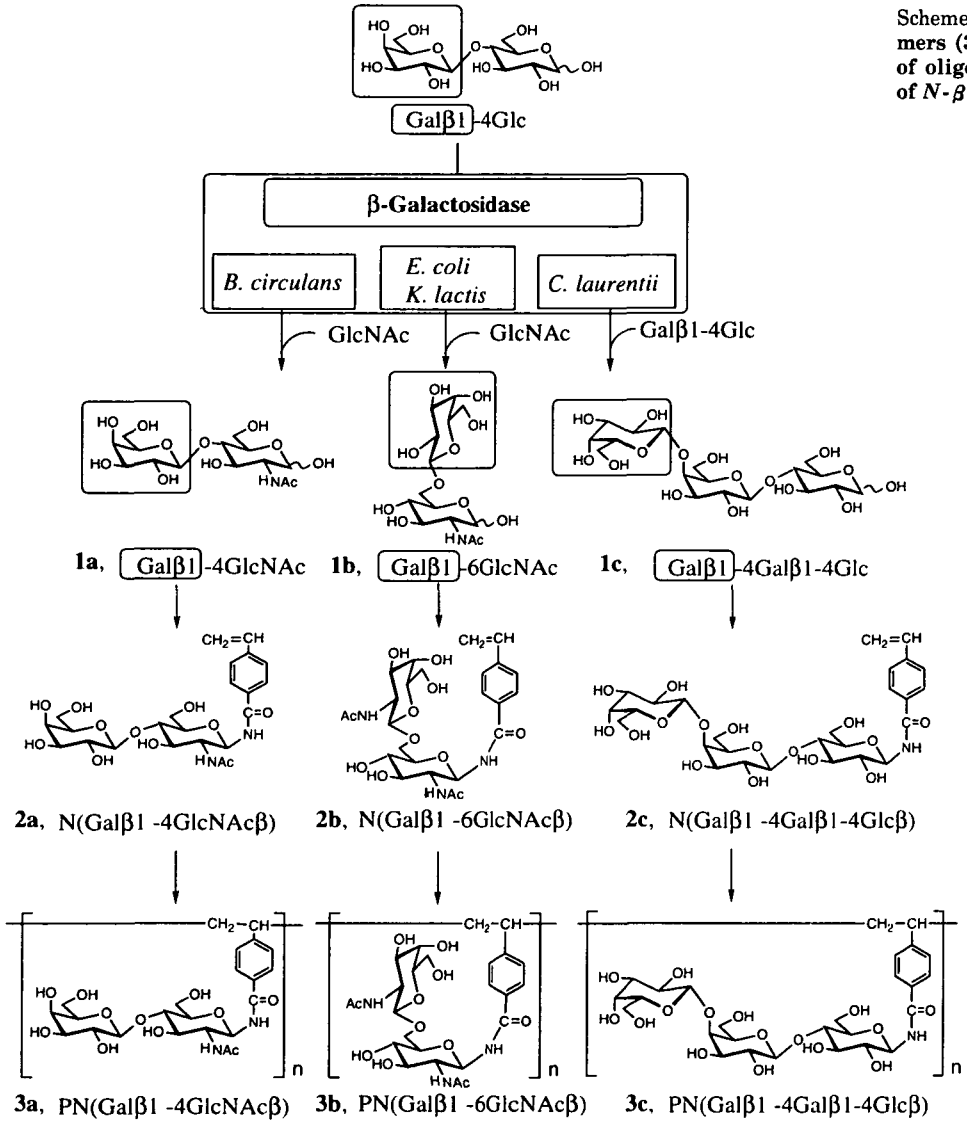


Scheme 1. **Synthetic routes to glycopolymers (3) *via* enzyme-catalyzed preparation of oligosaccharides (1) and polymerization of *N*-β-glycosidated styrene derivatives (2).**

Merck TLC plates precoated with silica gel 60. Preparative chromatography was carried out using a Yamazen preparative liquid chromatograph.

Materials—*N*-Acetyllactosamine and *N*-acetylisolactosamine were prepared as previously reported. 4′-Galactosyllactose was a gift from Nissin Sugar Mfg. (Tokyo). *p*-Vinylbenzoyl chloride was prepared from *p*-vinylbenzoic acid (Hokko Chemical Industry, Tokyo) according to the published procedure (*22*). Lectins used were purchased from Sigma.

2-Acetamido-4-O-(β-D-galactopyranosyl)-2-deoxy-N-(4-vinylbenzoyl)-β-D-glucopyranosylamine (2a): *N*-Acetyllactosamine (0.05 g, 0.14 mmol) was dissolved in water (5.0 ml) and ammonium hydrogen carbonate was added until a portion of the solid salt remained undissolved. The mixture was stirred in an open vessel at 37°C for 4 days. Ammonium hydrogen carbonate (the total amount, 8.9 g) was added at intervals to ensure saturation. TLC (ethyl acetate:acetic acid:methanol:water=4:3:3:1 in volume): R_f of *N*-acetyllactosamine=0.49 and of the amine derivative=0.38. When TLC indicated no further conversion, the mixture was diluted with water (30 ml) and concentrated to 5 ml. The residue was diluted to 50 ml with water and concentrated to 5 ml. This procedure was repeated twice.

Sodium carbonate (0.1 g) and methanol (1.0 ml) were added to a solution of the crude lactosylamine in water (1.0 ml). The mixture was stirred magnetically at 0°C for 2 h, and *p*-vinylbenzoyl chloride (0.10 ml, 0.7 mmol) in tetrahydrofuran (0.5 ml) was added. TLC (ethyl acetate:acetic acid:methanol:water=4:3:3:1 in volume): R_f of the product=0.71. After 5 h, the mixture was washed with chloroform (50 ml×3) to remove unreacted *p*-vinylbenzoyl chloride. The solution was concentrated to 5-10 ml and the crystallized product was chromatographed on a TSKgel HW-40S column (eluent, water). Fractions containing the product were collected and lyophilized. Yield was 0.05 g (64%). $[\alpha]_D^{25}-13.7°$ (*c* 0.2, Me_2SO).

^{1}H-NMR (D_2O) δ 1.83 (3H, s, $NHCOCH_3$), 3.40-3.80 (12H, m, from sugar), 4.36 (1H, d, $J_{1',2'}=7.6$ Hz, H-1′), 5.13 (1H, m, $J_{1,2}=9.5$ Hz, H-1), 5.30 {1H, d, $J=11.0$ Hz, CH_2=CH (*cis*)}, 5.82 {1H, d, $J=17.7$ Hz, CH_2=CH (*trans*)}, 6.69 (1H, dd, $J=11.0$ and 17.7 Hz, CH_2=C*H*),

7.46 and 7.58 (4H, d, $J=8.3$ Hz, C_6H_4). When Me_2SO-d_6 was used as the solvent, the following signals appeared: 8.20 (1H, d, $J=7.6$ Hz, N*H*COCH$_3$), 8.68 (1H, d, $J=8.1$ Hz, CONH).

^{13}C-NMR (D_2O) δ 22.3 (CH_3), 54.5 (C-2), 60.5 and 61.4 (C-6 and C-6′), 69.0–78.8 (C-3, C-5, C-2′, and C-5′), 79.8 (C-1), 82.1 (C-4), 103.3 (C-1′), 117.4 (*C*H$_2$=CH), 132.0 and 142.3 (*ipso*-phenyl), 126.9 and 128.2 (*meta*- and *ortho*-phenyl), 136.1 (CH$_2$=*C*H), 171.0 (C=O), 175.5 (C=O of sugar).

*2-Acetamido-6-O-(β-D-galactopyranosyl)-2-deoxy-N-(4-vinylbenzoyl)-β-D-glucopyranosylamine (**2b**):* Amination was carried out at 37°C for 4 days. TLC (ethyl acetate:acetic acid:methanol:water = 4:3:3:1 in volume): R_f of *N*-acetylisolactosamine = 0.43, of the amine derivative = 0.21, and of *p*-vinylbenzamido-β-*N*-acetyllactosamine = 0.75. Yield was 0.081 g (57%). $[\alpha]_D^{25}$, −10.6° (*c* 0.2, Me_2SO).

^{1}H-NMR (D_2O) δ 1.83 (3H, s, NHCOCH$_3$), 3.37–3.87 (12H, m, sugar), 4.10 (2H, d, H-1), 4.28 (1H, d, $J_{1',2'}=7.4$ Hz, H-1′), 5.13 (1H, m, $J_{1,2}=9.9$ Hz, H-1), 5.28 {1H, d, $J=11.0$ Hz, C*H*$_2$=CH (*cis*)}, 5.81 {1H, d, $J=17.7$ Hz, C*H*$_2$=CH (*trans*)}, 6.67 (1H, dd, $J=11.0$ and 17.7 Hz, CH$_2$=C*H*), 7.44 and 7.57 (4H, d, $J=8.3$ Hz, C_6H_4). ^{13}C-NMR (D_2O) δ 22.0 (CH_3), 54.4 (C-2), 61.1 (C-6′), 68.6–78.6 (C-3, C-6, C-2′, and C-5′), 79.5 (C-1), 103.3 (C-1′), 117.0 (*C*H$_2$=CH), 131.5 and 141.9 (*ipso*-phenyl), 126.5 and 128.0 (*meta*- and *ortho*-phenyl), 135.7 (CH$_2$=*C*H), 170.7 (C=O), 175.0 (C=O of sugar).

*4′-O-(β-D-Galactopyranosyl)-4-O-(β-D-galactopyranosyl)-N-(4-vinylbenzoyl)-β-D-glucopyranosylamine (**2c**):* Amination was conducted at 37°C for 4 days. TLC (ethyl acetate:acetic acid:methanol:water = 4:3:3:1 in volume): R_f of 4′-galactosyllactose = 0.25, of β-4′-galactosyllactosylamine = 0.14 and of *p*-vinylbenzamido-β-4′-galactosyllactose = 0.42. Yield was 0.11 g (87%). $[\alpha]_D^{25}$, +3.60° (*c* 1.0 in H_2O).

^{1}H-NMR (D_2O) δ 3.20–4.03 (18H, m, sugar), 4.37–4.32 (2H, m, $J_{1',2'}=J_{1'',2''}=7.8$ Hz, H-1′ and H-1″), 5.07 (1H, d, $J_{1,2}=9.2$ Hz, H-1), 5.30 {1H, d, $J=10.8$ Hz, C*H*$_2$=CH (*cis*)}, 5.81 {1H, d, $J=17.9$ Hz, C*H*$_2$=CH (*trans*)}, 6.80 (1H, dd, $J=10.8$ and 17.9 Hz, CH$_2$=C*H*), 7.45 and 7.67 (4H, d, $J=8.3$ Hz, C_6H_4). When Me_2SO-d_6 was used as the solvent, the following signal appeared: 8.60 (1H, d, $J=8.6$ Hz, CONH). ^{13}C-NMR (D_2O) δ 60.2-61.1 (C-6, C-6′, and C-6″), 68.7-78.7 (the other pyranose carbons), 79.9 (C-1), 103.0 (C-1′), 104.3 (C-1″), 117.0 (*C*H$_2$=CH), 131.9 and 141.9 (*ipso*-phenyl), 126.5 and 128.0 *meta*- and *ortho*-phenyl), 135.8 (CH$_2$=*C*H), 171.2 (C=O).

Polymerization—Prescribed amounts of the monomer and azobisisobutyronitrile were charged in a glass ampule and dissolved in dimethyl sulfoxide. The ampule was placed in a carbon dioxide-methanol bath, and the solution was frozen and degassed. The procedure was repeated three times. The ampule was sealed under reduced pressure and maintained in a thermostat at 60±0.05°C. It was then chilled and the solution was poured into an excess amount of cold methanol. The product was reprecipitated from its aqueous solution into methanol. The precipitate was dissolved in water, dialyzed in cellulose tubing (cut-off molecular weight, 3,500; diameter, 11 mm; thickness, 0.03 mm; Nacalai Tesque, Kyoto) against water for three days, and freeze-dried to give a white powdery polymer.

*Poly(p-vinylbenzamido-β-N-acetyllactosamine) (**3a**):* ^{1}H-NMR (D_2O) δ 1.15–1.90 (main chain -CH$_2$-CH-), 1.62 (NHCOCH$_3$), 4.39 (H-1′), 5.13 (H-1), 3.49–3.95 (other pyranose protons), 6.29 and 7.27 (phenyl), 8.38 (CONH). ^{13}C-NMR (D_2O) δ 22.2 (NHCOCH$_3$), 42.0 (broad, main chain CH$_2$-CH), 54.3 (C-2), 61.1 (C-6 and C-6′), 103.3 (C-1′), 68.8–76.7 (other pyranose carbons), 127.6 (*meta*- and *ortho*-phenyl), 131.1 and 148.0 (*ipso*- and *para*-phenyl), 169.3 (CO), 174.5 (NHCOCH$_3$).

*Poly(p-vinylbenzamido-β-N-acetylisolactosamine) (**3b**):* ^{1}H-NMR (Me_2SO-d_6) δ 1.64 (main chain -CH$_2$-CH-), 1.95 (NHCOCH$_3$), 5.32 (H-1), 3.35–4.79 (other pyranose protons), 6.43 and 7.41 (C_6H_4), 7.08 (NHCOCH$_3$), 8.32 (CONH). ^{13}C-NMR (Me_2SO-d_6) δ 22.6 (NHCOCH$_3$), ~40.0 (broad, main chain CH$_2$-CH), 60.4 (C-6, C-6′), 66.8–75.0 (other pyranose carbons), 77.7 (C-1), 103.5 (C-1′), 126.7 (*meta*- and *ortho*-phenyl), 135.0 and 149.2 (*ipso*- and *para*-phenyl), 165.5–171.1 (CO and NHCOCH$_3$).

*Poly(p-vinylbenzamido-β-4′-galactosyllactose) (**3c**):* ^{1}H-NMR (D_2O) δ 1.45 (main chain -CH$_2$-CH-), 5.06 (H-1), 3.79–3.54 (other pyranose protons), 6.43 and 7.37 (C_6H_4). ^{13}C-NMR (D_2O) δ 42.3 (broad, main chain CH$_2$-CH), 60.9–61.2 (C-6, C-6′, and C-6″), 68.9–78.9 (other pyranose carbons), 80.2 (C-1), 79.2 (C-4), 103.7 and 104.5 (C-1″), 127.6 (*meta*- and *ortho*-phenyl), 130.6, 150.3 (*ipso*- and *para*-phenyl), 168.0 (CO).

Assay of Inhibition of Lectin-Induced Hemagglutination by Glycopolymers (23)—Erythrocyte suspension (20 μl) was added to twofold dilutions (20 μl) of lectins in 96-well microtiter U-plates and incubated for 1 h. The minimum concentration of lectin required for agglutination of erythrocytes was determined, and a 4-fold greater concentration was used for the following inhibition assay.

Twofold dilutions (20 μl) of oligosaccharides and glycopolymers were prepared in 96-well plates. An aliquot (20 μl) of the lectin solution was added to each well and the plates were incubated at 30°C for 1 h. An erythrocyte suspension (40 μl) was added to the hole and incubation was continued at 30°C for 1 h. Agglutination of erythrocytes was carefully observed and the minimum concentrations of oligosaccharides and glycopolymers required to inhibit erythrocyte agglutination were determined.

RESULTS AND DISCUSSION

Synthesis of Oligosaccharide-Carrying Styrene Derivatives and Artificial Glycoconjugate Polymers—The three enzymatically synthesized oligosaccharides, *N*-acetyllactosamine (**1a**), *N*-acetylisolactosamine (**1b**), and 4′-galactosyllactose (**1c**) were converted to the styrene derivatives *via* a two-step procedure. The yield was 64% for **2a**, 57% for **2b**, and 87% for **2c**.

The *N*-linked β-anomeric bond between glycosyl and vinylbenzamido residues was confirmed by the following ^{1}H- and ^{13}C-NMR assignments. The anomeric protons showed lower chemical shifts (**2a**, δ 5.13 ppm; **2b**, δ 5.13 ppm; **2c**, δ 5.07 ppm) and larger coupling constants (**2a**, $J_{1,2}=9.5$ Hz; **2b**, $J_{1,2}=8.3$ Hz; **2c**, $J_{1,2}=9.2$ Hz) than the other *O*-β-linked anomeric protons (**2a**, δ 4.36 ppm, $J_{1',2'}=7.6$ Hz for H-1′; **2b**, δ 4.28 ppm, $J_{1',2'}=7.4$ Hz for H-1′; **2c**, δ 4.37–4.32 ppm, $J_{1',2'}=J_{1'',2''}=7.8$ Hz for H-1′ and H-1″). In the ^{13}C-NMR spectra, the *N*-β-linked C-1β signals were distinct in having higher chemical shifts (**2a**, δ 79.8 ppm;

2b, δ 79.5 ppm; **2c**, δ 79.9 ppm), compared to those of O-β-linked C-1′ signals. The other ^{1}H- and ^{13}C-NMR assignments are summarized in the experimental section. No other minor product suggesting N-α-linkage and benzoyl ester linkage was detected. The N-glycosidation proceeded stereo specifically in one flask to give only the β-glycoside without any protection and deprotection steps.

Table I summarizes the results of homopolymerization and copolymerization with acrylamide, with 2,2′-azobisisobutyronitrile as an initiator, in dimethyl sulfoxide at 60°C. Copolymer composition was estimated from the ^{1}H-NMR area ratio of amide, phenyl, anomer H-1, and main chain methylene proton signals. These homopolymers (**3a**, **3b**, and **3c**) were soluble in water and dimethyl sulfoxide, and insoluble in N,N-dimethylformamide, pyridine, methanol, and other common organic solvents.

Interaction between Lectins and Glycopolymers—The following data on lectins were collected from the literature. RCA_{120} (MW 1.2×10^5) consists of two A and two B chains, and is specific for the β-Gal unit (*24*). PNA (MW 1.2×10^5) isolated from peanuts (*Arachis hypogaea*) consists of four equal-sized subunits (*25*). It is specific for the disaccharide Galβ1-3GalNAc, and the configuration of the terminal Gal hydroxyls plays a key role. ECA (MW 5.68×10^4) from seeds of coral tree (*Erythrina cristagalli*) consists of two subunits (*26*). It is specific for Galβ1-4GlcNAc, in which C′-4 (Gal-) and C-2 (GlcNAc) are important binding factors. It also binds to the terminal Gal of Galβ1-4Glc. Wheat germ agglutinin (WGA, MW 3.6×10^4) is composed of two similar polypeptide chains, and is specific to GlcNAc and its β-1,4 linked oligomers (*27, 28*). DSA (MW 8.6×10^4) obtained from Jimson weed specifically binds tetra antennary β-1,4-linked GlcNAc oligosaccharides (*29*).

Table II summarizes the inhibitory effects of glycopolymers on the hemagglutination of human A-type blood cells by lectins. The minimum concentrations of glycopolymers are listed as molarity (mol/liter) of oligosaccharide unit and as weight concentration (mg/ml) of the polymer. Lower values mean stronger inhibition ability. Inhibition was observed between the glycopolymers (**3a**, **3b**, and **3c**) and the galactose-binding lectins (ECA, PNA, and RCA_{120}) and between the glycopolymers (**3a** and **3b**) and the N-acetylglucosamine binding lectin (WGA and DSA). The qualitative results on hemagglutination are consistent with the reported specificities of these lectins for glycoproteins, glycolipids, and oligosaccharides.

The homopolymer **3a** carrying the Galβ1-4GlcNAc unit showed effective inhibition ($\sim10^{-6}$) of hemagglutination by ECA and RCA_{120}. The activity towards RCA_{120} lectin was about 10^3 times stronger than that of the oligosaccharide itself (**1a**) and about 10^2 times stronger than that of the copolymer. The cluster effect was remarkable. On the other hand, the activity towards ECA, which is specific to Galβ1-4GlcNAc, was only 10 times stronger than that of

TABLE I. **Polymerization[a] of p-vinylbenzamido-β-oligosaccharide (2a, 2b, and 2c).**

Run no.	Monomer (g)	Acrylamide (g)	Feed[b] (mol fr.)	AIBN (mol %)	Me_2SO (ml)	Yield (%)	Copolymer (mol fr.)	M_n[c]$\times10^{-6}$	M_w/M_n
13	**2a** 0.05	0.05	0.12	1.0	0.7	44	0.10		
15	0.06	0	1.0	1.0	0.8	45	1.0	1.2	1.35
19	0.05	0.05	0.12	0.5	0.6	46	0.25		
33	**2b** 0.10	0	1.0	1.0	0.7	72	1.0		
11	0.10	0.40	0.10	0.5	2.0	56	0.11	2.0	1.7
12	**2c** 0.10	0.10	0.12	0.5	1.0	85	0.10	5.8	1.3
14	0.10	0	1.0	1.0	1.2	56	1.0	4.9	1.7

[a]At 60°C, 15 h. [b]Mole fraction of p-vinylbenzamido-β-oligosaccharides. [c]By SEC using H_2O eluent and pullulan standard.

TABLE II. **Inhibition of hemagglutinating activity of lectins by glycopolymers.[a]**

Inhibitor	Minimum inhibitory concentration, M[b] (mg/ml)				
	RCA_{120}[c]	PNA[d]	ECA[e]	WGA[f]	DSA[g]
Galβ1-4GlcNAc					
Homopolymer **3a**	2×10^{-6} (0.0008)	3×10^{-4} (0.156)	6×10^{-6} (0.003)	5×10^{-3} (2.5)	1×10^{-2} (9.0)
Copolymer[i]	1×10^{-4} (0.16)	5×10^{-4} (0.078)	6×10^{-5} (0.078)	N.I.[h]	4×10^{-3} (5.0)
Monomer **2a**	6×10^{-4} (0.31)	2×10^{-2} (10.0)	3×10^{-4} (0.16)	2×10^{-2} (10.0)	4×10^{-2} (20.0)
Sugar **1a**	2×10^{-3} (0.63)	3×10^{-2} (10.0)	3×10^{-5} (0.16)	N.I.	N.I.
	RCA_{120}[c]	PNA[d]	ECA[e]	WGA[f]	DSA[g]
Galβ1-6GlcNAc					
Homopolymer **3b**	1×10^{-3} (0.63)	1×10^{-2} (5.0)	2×10^{-2} (10.0)	N.I.	N.I.
Copolymer[j]	1×10^{-2} (10.0)	1×10^{-2} (10.0)	3×10^{-3} (2.5)	5×10^{-5} (0.04)	2×10^{-2} (15.0)
Monomer **2b**	2×10^{-4} (0.10)	3×10^{-4} (0.15)	4×10^{-4} (0.20)	N.I.	N.I.
Sugar **1a**	2×10^{-3} (0.63)	6×10^{-4} (0.25)	N.I.	N.I.	N.I.
	RCA_{120}[c]	PNA[d]	ECA[e]	WGA[f]	DSA[g]
Galβ1-4Galβ1-4Glc					
Homopolymer **3c**	2×10^{-3} (1.30)	6×10^{-5} (0.039)	4×10^{-3} (2.5)	N.I.	N.I.
Copolymer[k]	2×10^{-4} (0.31)	1×10^{-5} (0.02)	1×10^{-4} (0.16)	N.I.	N.I.
Monomer **2c**	3×10^{-2} (20.0)	4×10^{-3} (2.5)	8×10^{-3} (5.0)	N.I.	N.I.
Sugar **1c**	3×10^{-2} (20.0)	5×10^{-3} (2.5)	5×10^{-3} (2.5)	N.I.	N.I.

[a][Lectin] = 4 × [minimum concentration required for hemagglutination]. [b]Molarity (mol·liter^{-1}) of oligosaccharide unit. [c]*Ricinus communis*, 3.8 μg/ml. [d]*Arachis hypogaea*, 1.0 mg/ml. [e]*Erythrina cristagalli*, 0.4 μg/ml. [f]*Triticum vulgaris*, 0.4 μg/ml. [g]*Datura stramonium*, 0.78 μg/ml. [h]N.I., not inhibited by 20 mg/ml (0.1-0.04 mM). [i]Sugar:acrylamide = 0.10:0.90. [j]Sugar:acrylamide = 0.25:0.75. [k]Sugar:acrylamide = 0.11:0.89.

the oligosaccharide itself.

The homopolymer **3b** carrying the Galβ1-6GlcNAc unit had low inhibition ability (10^{-2}-10^{-4}) towards ECA, RCA, and PNA lectins, lower than those of the oligosaccharide itself (**1b**) and the corresponding styrene monomer (**2b**). A possible explanation is that the Galβ1-6GlcNAc units along the glycopolymer **3b** were subject to steric hindrance and were less able to approach the binding pockets of these lectins. The homopolymer **3c** carrying the Galβ1-4Galβ1-4Glc unit showed relatively high affinity ($\sim 10^{-5}$) for PNA. The Galβ1-4Galβ residue of **3c** was recognized by PNA, which is specific for Galβ1-3GalNAc.

Figure 1 compares the inhibitions of galactose-binding lectin-induced hemagglutination by the glycopolymers which carry 1-4-linked disaccharide **3a** and trisaccharides **3c**, together with the previously reported lactose-carrying glycopolymers PN(Galβ1-4Glcβ) (**3d**). Stronger inhibitions are indicated by longer bars. In Fig. 1(a), PN(Galβ1-4GlcNAcβ) (**3a**) showed the highest activity for RCA_{120}.

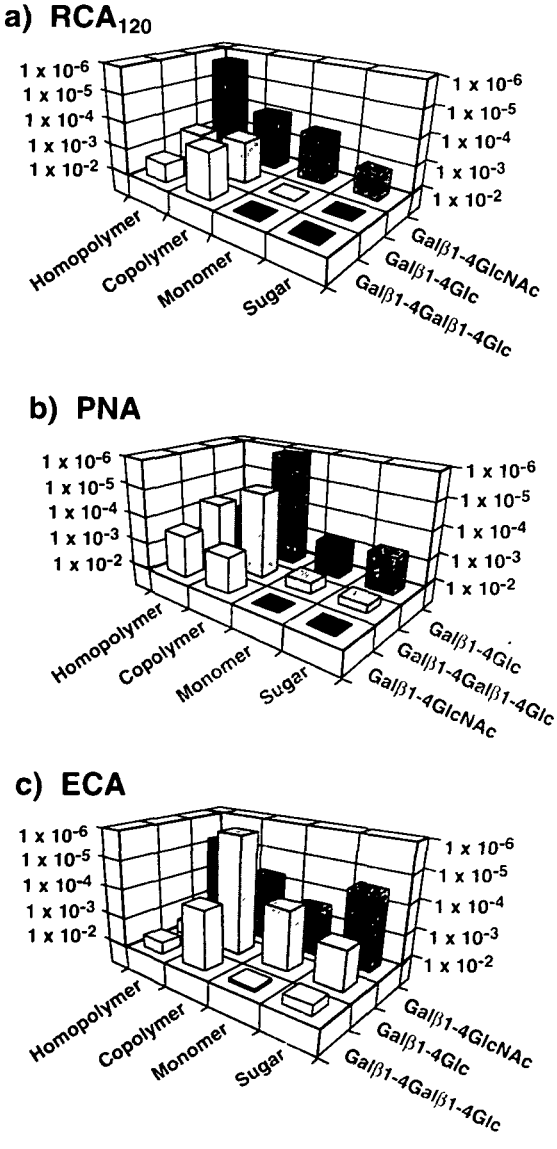
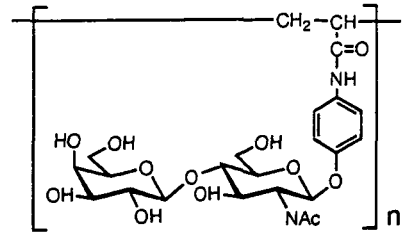


Fig. 1. **Inhibition effect of glycopolymers on hemagglutination by (a) RCA_{120}, (b) PNA, and (c) ECA lectins.**

The RCA_{120} binding ability decreased in the order of PN(Galβ1-4GlcNAcβ) (**3a**) > PN(Galβ1-4Glcβ) (**3d**) > PN(Galβ1-4Galβ1-4Glcβ) (**3c**). The corresponding oligosaccharides themselves showed almost no binding activity. In Fig. 1, (b) and (c), the binding activities of these copolymers were higher than those of the corresponding homopolymers. The results will be discussed in the following section.

Comparison of N-Acetyllactosamine-Carrying Polymers Having Different Linkages—Other types of glycopolymers carrying the Galβ1-4GlcNAc, Galβ1-3GlcNAc, and Galβ1-6GlcNAc units were reported by Matsuoka and Nishimura (*30*). The corresponding neopentyl glycosides were synthesized by applying fine chemical glycosidation methods, and the resulting monomeric substances were copolymerized with acrylamide. *Erythrina corallodendron* lectin was found to bind the *N*-acetyllactosamine-carrying glycopolymer most strongly among these neopentyl glycopolymers.

We also previously synthesized another type of glycopolymer carrying the *N*-acetyllactosamine residue, PAP(Galβ1-4GlcNAcβ) (**4**), which has an *O*-linked β-anomeric phenyl aglycon along a polyacrylamide backbone unit (*31*). It is of interest to compare the recognition ability of the glycopolymers **3a** and **4**, which have different linkage modes and different backbones. For hemagglutination induced by DSA lectin, the minimun inhibitory concentration of **3a** was 1×10^{-2} M, and that of **4** was 4×10^{-6} M. The *O*-β-glycoside-bearing polymer **4** showed about 3×10^3 times stronger inhibition ability than the *N*-β-glycoside-bearing polymer **3a** (Scheme 2).



Scheme 2. **PAP(Galβ1-4GlcNAcβ), 4.**

The stronger interaction of **4** with DSA was also confirmed by the double diffusion test. Sharp precipitation bands appeared on the gel between the wells of DSA and the *O*-β-glycoside-bearing polymer **4**, but only ambiguous precipitation bands appeared for the *N*-β-glycoside-bearing polymer **3a**.

Inhibition data for the corresponding monomer to **4** are indispensable for a discussion of the difference between *O*- and *N*-glycosides as well as the difference between the aglycon structures. Unfortunately, all of the monomer was used for polymerization and so inhibition data are not available. Elucidation of the interactions of these *O*- and *N*-glycosides with DSA will require further study.

Discussion of Lectin-Glycopolymer Interactions—Recently, Kajiwara *et al.* (*31*) investigated the small-angle X-ray diffraction and light scattering of these types of glycopolystyrene homopolymers. The data could be explained by assuming that these glycohomopolymers have rod-like conformations in water. The oligosaccharide units are attached to every repeating unit along the polymer chain, and the hydrophobic polystyrene main chains are buried inside the molecule to form a hydrophobic core that

is sheltered from water. Hence, the oligosaccharide chains tend to gather on the outside of the polymer. In other words, the glycohomopolymers have numerous multi-antennary or clustered oligosaccharide terminals, which enhance the binding to some lectins. This effect was especially important in the cases of WGA and DSA lectins, which recognize multi-antennary *N*-acetylchito-oligosaccharides.

In some of the combinations between lectins and polymers listed in Table II and Fig. 1, the copolymers inhibited hemagglutination more strongly than the homopolymers did. In copolymers, side chain oligosaccharides are separated by acrylamide units. When the oligosaccharides are located at a suitable distance, some lectins can interact much more effectively with the glyco-copolymers. The amount of unavailable oligosaccharides that buried in the polymer was also reduced. This corresponds to the negative cluster effect proposed by Nishimura *et al.* (*33*).

Kiessling *et al.* (*34*) discussed the lectin-binding of glycopolymers composed of rigid polymeric backbones. The multivalent ligands along the rigid polymeric backbone interacted weakly with lectins, which was ascribed to incompatible relative orientation and incompatible spacing of the saccharide units. However, this is not applicable to the present flexible glycopolystyrene backbones (*35*).

In addition to the cluster and negative cluster effects, another polymeric effect was discussed by Whitesides *et al.* (*10*). When macromolecular chains of glycopolymers occupy the space between a red blood cell and a lectin, a large steric hindrance is induced between the cell and the protein. As a result, apparent effective binding of glycopolymers with lectins was brought about. The steric effect was important not only for homopolymers, but also for copolymers.

Artificial glycoconjugate polymers are useful tools to investigate interactions with lectins and also promising biomaterials for the application of oligosaccharide-recognizing ability.

The authors are grateful to Nisshin Sugar Mfg. Co., Ltd. (Tokyo) and Hokko Chemical Industry Co., Ltd. (Tokyo) for gifts of 4′-galactosyllactose and *p*-vinylbenzoic acid, respectively.

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