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Simple synthesis of sialyllactose-carrying polystyrene and its binding with influenza virus

Akiko Tsuchida[1], Kazukiyo Kobayashi[1]*, Noritaka Matsubara[2], Tsukasa Muramatsu[3], Takashi Suzuki[3], and Yasuo Suzuki[3]

[1]*Graduate School of Engineering, Nagoya University, Chikusa-ku, Nagoya 464-8603, Japan*
[2]*Snow Brand Milk Products Co., Ltd., Kawagoe, Saitama 350-11, Japan*
[3]*Department of Biochemistry, University of Shizuoka School of Pharmaceutical Sciences, Yada, Shizuoka 422-8526, Japan*

Glycoconjugate polystyrenes bearing sialyllactose moieties were prepared via a simple method from a mixture of α2-6 and α2-3 linked sialyllactose isomers of bovine milk origin. The reducing end of sialyllactose was converted to an amino function with ammonium hydrogen carbonate and then coupled with *p*-vinylbenzoyl chloride. The resulting styrene derivative substituted with sialyllactose via an amide linkage was polymerized with ammonium peroxodisulfate and *N,N,N',N'*-tetramethylethylenediamine in water at 30 °C. The interaction of the glycopolymer with influenza A and B viruses was investigated by three different methods. The glycopolymer inhibited the hemagglutination of influenza A virus (PR/8/34) and its activity was 10^3 times higher than that of the oligosaccharide itself. The cytopathic effect of virus-infected MDCK (Madine-Darby canine kidney) cells was inhibited by the glycopolymer. The homopolymer showed 10^2 times higher inhibitory activity than naturally-occurring fetuin. It was also found that various viruses could be trapped by the glycopolymer adsorbed on a polystyrene surface. The inhibitory and trapping activities of the glycopolymers were correlated with the sialyl linkage specificities of the virus strains.

Keywords: **glycopolymer, influenza virus, sialyllactose, inhibitor**

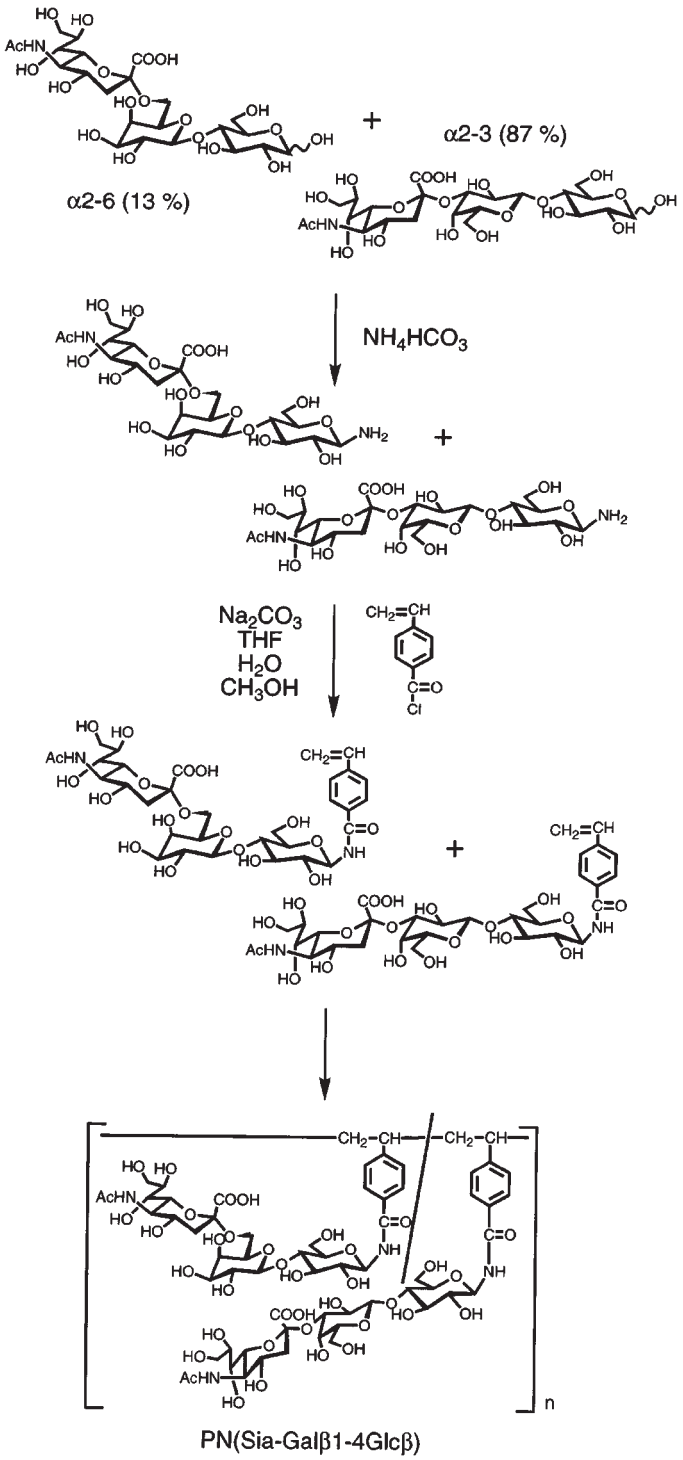
Introduction

Sialyl oligosaccharides are essential components of the glycoproteins and glycolipids of cell membranes [1–3]. They are exposed to the external environment and play a major role in the negative charge of cells. These sialyl oligosaccharides are indispensable in various biological recognition events. They serve as ligands for enzymes, hormones, toxins, lectins, bacteria, and viruses, as differential antigens and tumor antigens, and as signals for the growth of nervous tissues. Recently, increasing attention has been paid to the fine-organic synthesis [4,5] of sialyl oligosaccharides and to the simple synthesis of their analogs or mimics [6–8] as tools for clarifying these biological and pathological processes.

The recognition ability of glyco-signals is known to be much enhanced when several oligosaccharide chains are aligned so as to induce-fit the binding sites of a receptor [9]. In this respect, polymeric materials carrying oligosaccharide moieties as pendant groups are of interest, because multiantennary or clustered oligosaccharides protrude from the polymer backbone [10–14]. Glycopolymers carrying monosialic acids with O-, S-, and C-glycoside linkages have been prepared by several groups [15–22]. These syntheses required multi-step procedures including protection and deprotection steps. Little has been reported on the synthesis of glycopolymers carrying sialyl oligosaccharides, except for enzymatic synthesis involving sialyltransferase on a galactosylated glycopolymer [23].

The present paper reports the simple synthesis of sialyllactose-carrying polystyrene via the convenient procedure presented in Scheme 1 [24,25]. Among its possible applications as a ligand for various bacterial strains [26] and selectin families [27], this paper focuses on the binding of the glycopolymer with influenza viruses. The interaction between an influenza virus and a host cell is mediated by a viral spike glycoprotein, hemagglutinin (HA), that recognizes the terminal Neu5Acα2-3Gal and/or Neu5Acα2-6Gal linkages in glycoproteins and glycolipids on cell-surface receptors [28–31]. It was reported that sialyllactose bound to influenza viruses more strongly than sailic acid itself [31]. The interaction of sialyllactose-carrying glycopolymers with viruses was investigated by means of inhibition of hemagglutination [32,33], inhibition of the cytopathic effect of Madine-Darby canine kidney (MDCK) cells [34,35], and

*To whom correspondence should be addressed. Tel: +81-52-789-2488; Fax: +81-52-789-2528; E-mail: kobayash@mol.nagoya-u.ac.jp



Scheme 1. A convenient synthesis of poly(p-vinylbenzoyl-β-sialyllactosylamine)

trapping of influenza viruses by the present glycopolymer immobilized on microtiter plates.

Materials and methods

General

NMR spectra were recorded with a Varian VXR-500 Fourier transform NMR spectrometer. Thin layer chromatography (TLC) was carried out on Merck TLC plates precoated with silica gel 60. Preparative chromatography was carried out with a Yamazen preparative liquid chromatograph. Sialyllactose was a gift from Snow Brand Milk Products Co., Ltd. (Japan).

N-p-Vinylbenzoyl-β-sialyllactosylamine

Sialyllactose was isolated [36] from bovine milk as a mixture of α(2-3) and α(2-6) linked isomers (6.5 : 1). A mixture of sialyllactose (100 mg, 0.16 mmol) and ammonium hydrogen carbonate in water (5 mL) was stirred in an open vessel at 37°C for 3 days. The ammonium hydrogen carbonate (total amount, 6.5 g) was added at intervals to ensure saturation. TLC (ethyl acetate : acetic acid : methanol : water = 4 : 3 : 3 : 2 in volume): R_f of sialyllactose = 0.40 and β-sialyllactosylamine = 0.18. When TLC indicated no more conversion, the mixture was diluted with water (50 mL) and then concentrated to 5 mL in a rotary evaporator. Excess ammonium hydrogen carbonate was removed by repeating the following procedure twice: dilution of the residue to 50 mL with water and then concentration of the solution to 5 mL [37].

Sodium carbonate (0.2 g) and methanol (3.0 mL) were added to the resulting crude sialyllactosylamine in water (5.0 mL). The mixture was stirred magnetically at 0°C for 1 h, and then *p*-vinylbenzoyl chloride (0.26 mL, 1.8 mmol) in tetrahydrofuran (3.0 mL) was added. TLC (ethyl acetate: acetic acid: methanol: water = 4 : 3 : 3 : 2 in volume): R_f of β-sialyllactosylamine = 0.18 and the product = 0.60. After 5 h, the mixture was washed with chloroform (30 mL × 3) to remove unreacted *p*-vinylbenzoyl chloride. The solution was concentrated to 5–10 mL and then the product was chromatographed on a Cellulofine A-500 column (DEAE-ion exchanger) (eluent, gradient of 0–0.1N aqueous sodium chloride solution). The fractions containing **1** were collected, desalted, and then lyophilized. The yield was 108 mg (88%).

^{1}H-NMR (D_2O) δ 1.65 (t, 1H, J = 12.5 Hz, H-3ax), 1.88 (CH_3), 2.60 (dd, 1H, J = 12.0 and 4.0 Hz, H-3eq), 3.43-3.97 (m, 20H, from sugar), 4.41 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H′−1), 5.07 (m, 1H, $J_{1,2}$ = 9.0 Hz, H−1), 5.29 {d, 1H, J = 10.9 Hz, CH_2=CH-(cis)}, 5.82 {d, 1H, J = 17.9 Hz, CH_2=CH-(trans)}, 6.70 (dd, 1H, J = 10.9 and 17.9 Hz, CH_2=CH-), and 7.58 and 7.90 (d, 4H, J = 8.2 Hz, C_6H_4).

^{13}C-NMR (D_2O) δ 22.1 (CH_3), 40.1 (C″−3), 51.8 (C″−5), 60.7 (C-6), 61.1 (C′−6), 62.7 (C″−9), 67.6-77.8 (other carbons from sugar), 79.9 (C−1), 99.9 (C″−2), 102.7 (C′−1),

116.9 (CH_2=CH-), 132.2 and 135.8 {phenyl-(*ipso*)}, 126.5 and 128.1 {phenyl-(*meta* and *ortho*)}, 141.8 ((CH_2=*C*H-), 171.5 (-NHCO-), 173.9 (-NH*C*OCH_3), and 175.1 (-COOH).

Polymerization

A solution of the sialyllactose-substituted styrene monomer (0.1 g, 0.13 mmol) in 1.0 mL of deionized water was degassed with an aspirator, and then *N,N,N′,N′*-tetramethylethylenediamine (TEMED) (6.3 μL) and ammonium peroxodisulfate (APS) (2.5 μg) were added. The mixture was stirred at room temperature for 3 h. The product was precipitated by pouring the aqueous solution of it into methanol. The precipitate was dissolved in water, dialyzed in a cellulose tube (cut-off molecular weight, 3500; diameter, 11mm; thickness, 0.03 mm; Nacalai Tesque, Kyoto, Japan) against water for 3 days, and then freeze-dried to give a white powdery polymer. ^{1}H-NMR (D_2O) δ 1.92 (main chain CH_2-CH-), 2.11(CH_3), 3.70-4.22 (other protons from sugar), 5.25 (H-1), and 6.61 and 7.58 (C_6H_4).

Materials for the bioassay

Influenza A and B virus isolates were grown in the allantoic sacs of 10-day-old embryonated eggs and then purified by sucrose-density-gradient centrifugation. The number of viral hemagglutination units (HAU) was determined at 4 °C as described previously [32]. Madine-Darby canine kidney (MDCK) cell monolayers were propagated in an Eagle's minimum essential medium (EMEM) containing 10% fetal calf serum. Rabbit anti-(influenza virus) antibodies were raised against whole A/PR/8/34 (H1N1), A/Memphis/1/74 (H3N2), and B/Lee/40 viruses grown in eggs [35].

Inhibition of hemagglutination [33]

The titer of the stock solution of influenza virus (A/PR/8/34) was determined by serial dilution of 25 μL of the virus solution in 12 wells of a 96-well microtiter plate, each containing 25 μL of 0.02% (W/V) gelatin-PBS. A suspension of chicken erythrocytes (0.5%) in PBS (25 μL) was then added to each well. After 1 h at 4 °C, the lowest concentration of virus that agglutinated the erythrocytes was determined by visual inspection. One HA unit (HAU) was defined as the highest dilution of the virus suspension giving complete agglutination of the erythrocytes and a 4 HA unit solution was used for the following inhibition test.

The lowest concentration of an inhibitor that prevented the agglutination of red blood cells in the presence of virus was then determined. A stock solution of the inhibitor in PBS (25 μL) was serial diluted in 12 microtiter plate wells, each containing 25 μL of 0.02% gelatin-PBS. A suspension of A/PR/8/34 (25 μL) virus at a concentration of 2^2 HAU was then added to each well. After 1 h at 4 °C, a suspension (100 μL) of chicken erythrocytes (0.5%) in PBS was added to each well with mixing. After 1 h at 4 °C, the lowest concentration of inhibitor that inhibited the agglutination of erythrocytes was determined. Sialyllactose was used as a reference compound.

Neutralization of influenza viruses by glycopolymers [34,35]

MDCK cell monolayers were inoculated with EMEM containing 0.2% BSA and approximately 100 $TCID_{50}$ (50% tissue-culture infectious dose) of A/PR/8/34 in the presence of the glycopolymer (0.01-10000 μg/mL) at 34.5 °C for 5 h. After removal of the inoculum, the monolayers were washed three times with EMEM containing 0.2% BSA and then incubated in 100 μL of the medium at 34.5 °C for 20 h. The progress of the viral-induced cytopathic effect (CPE) was monitored by light microscopy. As a control as to viral-induced CPE, cells were inoculated with the virus in the presence of viral antibodies diluted with EMEM (1 : 500). The activity of lactate dehydrogenase (LDH) released from MDCK cells was determined by the slightly modified colorimetric assay previously described and was used as a measure of virus neutralization. The medium (12.5 μL) was diluted with PBS (1 : 4) and then mixed with 50 μL of an LDH reagent (Shino-test, Japan). The mixture was incubated at 37 °C for 10 min and the reaction was stopped by the addition of 100 μL of 0.5 M HCl. The absorbance was measured at 550 nm (reference, 630 nm). The assays were carried out in duplicate.

Trapping of influenza viruses by the immobilized glycopolymer in microtiter wells

The glycopolymer was adsorbed on the polystyrene surface of a microtiter plate (Immuno plate MaxiSorp, Nunc, Denmark) by treating the wells with a polymer solution (10 μg/mL) in PBS (100 μL/well) at 37 °C for 12 h. The solution was removed and then the wells were treated with 200 μL of PBS containing 1% of BSA at 37 °C for 5 h to block the remaining hydrophobic surface with BSA. The wells were then washed with PBS.

Suspensions (50 μL) of influenza viruses A/PR/8/34, A/Memphis/1/74, and B/Lee/40 (2^{10} HAU) were diluted serially two-fold with PBS containing 1% (w/v) BSA. The suspensions were added to the polymer-coated microtiter wells, followed by incubation at 4 °C for 12 h, and then the wells were washed with PBS five times. The plates were incubated at 37 °C for 2 h with each rabbit anti-influenza virus antibody diluted with PBS (1 : 1000) containing 1% (w/v) BSA, and then treated with horseradish peroxidase-conjugated Protein A (Organon Teknika N. V. Cappel Products) for the rabbit anti-influenza virus antibodies diluted with PBS (1 : 1000). The plates were washed five times with PBS, and then the virions bound to the glycopolymer were detected with a 100 μL solution comprising 4 mg of *o*-phenylenediamine (OPD) and 0.01% H_2O_2 in 100 mM phosphate-citric acid buffer (pH 5.0). The reaction was stopped with 100 μL of 4 N H_2SO_4, and viral binding activ-

ity was determined as color development at 492 nm, with a reference wavelength at 630 nm. As negative controls, each influenza virus was added to BSA-immobilized wells instead of the glycopolymer-immobilized wells.

Results and discussion

Synthesis of a sialyllactose-carrying polymer

Sialyllactose was isolated as a mixture of α(2-3) and α(2-6) linked isomers (molar ratio, 6.5 : 1) from bovine milk. As shown in Scheme 1, an amino function was derived from the reducing terminal of sialyllactose with ammonium hydrogen carbonate and then allowed to react with *p*-vinylbenzoyl chloride without isolation of the intermediate glycosylamine. The two-step procedure was carried out practically in one flask with an 88% yield. The monomeric product *N*-*p*-vinylbenzoyl-β-sialyllactosylamine, was soluble in water, dimethyl sulfoxide, *N,N*-dimethylformamide, and pyridine.

The structure of the *N*-*p*-vinylbenzoyl-β-sialyllactosylamine monomer was confirmed by the ^{1}H- and ^{13}C-NMR spectra. The signals of the α(2-3) linked sialyllactose main component were assigned, and are summarized earlier. An N-linked β-anomeric glucopyranose proton signal (H-1) appeared at δ 5.07 ppm ($J_{1,2}$=9.0 Hz). The N-β-linked proton showed a lower chemical shift and a larger coupling constant than the O-β-linked galactopyranose proton signal (H-1′) at δ 4.41 ppm ($J_{1',2'}$ = 7.8 Hz). ^{13}C-NMR anomeric signals of N-β-glucopyranosyl, O-β-galactopyranosyl, and α-5-acetylneuraminic acid moieties were observed, respectively, at 79.9 ppm (C-1), 102.7 (C″−1), and 99.9 ppm (C″−2). No other minor products such as N-α-linked glycosyl compounds and vinylbenzoate ester compounds were detected. The N-glycosidation proceeded stereospecifically in one flask to give only the β-glycoside without any protection and deprotection steps. It is important to note that this simple method could be applied to biologically important oligosaccharides containing sialic acid.

Table 1 summarizes the homo- and copolymerization of the *N*-*p*-vinylbenzoyl-β-sialyllactosylamine monomer with acrylamide using ammonium peroxodisulfate and *N,N,N′,N′*-tetramethylethylene diamine as the initiators in water at 30 °C. The copolymer composition was estimated from the intensities of ^{1}H-NMR signals of the respective protons: the amide and phenyl protons, H-1, and main chain methylene protons. The sialyllactose- bearing homopolymer were soluble in water and dimethyl sulfoxide, but insoluble in pyridine and methanol. The molecular weights and distributions of the polymers were estimated by SEC in phosphate buffer.

Inhibition of hemagglutination

Influenza viruses are known to interact with erythrocytes to induce hemagglutination or the formation of extended cross-linked gels. Various glycosylated substances carrying sialyl oligosaccharides inhibit the hemagglutination by binding to the hemagglutinin proteins on the virus virions. The present glycopolymer inhibited the hemagglutination of influenza virus A/PR/8/34 (H1N1) at the concentration of 1.3×10^{-6} M (or 1×10^{-3} mg/mL). The inhibitory abilities of sialyllactose itself and the corresponding lactose-carrying polystyrene were not so strong: their inhibitory concentrations were higher than 2×10^{-3} M (or > 1 mg/mL). Sialyllactose-carrying glycopolymers were found to bind the viruses about 10^3 times stronger than sialyllactose itself. The sialyllactose-substituted styrene monomer also showed rather strong binding ability as to viruses at the concentration of 2.0×10^{-5} M. The presence of hydrophobic aglycon enhanced the binding with hemagglutinin proteins as reported [38].

The inhibitory concentrations of the acrylamide-copolymers are plotted as a function of the copolymer composition in Figure 1. Even the copolymer with 0.04 mol fraction (or 0.31 weight fraction) of glycosylated units showed rather strong inhibition at 10^{-5} M^{-1}. Because hemagglutination requires contact between the surface of the virions and the erythrocytes, the presence of a solvent-swollen polymer on the surface of virions would hinder their contact. The con-

Table 1. Homo- and copolymerization[a] of p-vinylbenzoyl-β-sialyllactosylamine (**1**).

Run no.	*mg of* **1**	*mg of acrylamide*	*mol fr. of* **1** *in feed*[b]	*mg of AP*[c]	*mL of TEMEDA*[c]	*mL of* H_2O	*time, h*	*yield, %*	*mol fr. of* **1** *in copolymer*	M_n[d] $\times 10^4$	$\frac{M_w}{M_n}$
28	100.0	0	1.0	2.5	12.5	1.0	3.0	72	1.00	3.5	2.1
30	50.0	2.0	0.73	2.5	12.5	0.5	5.0	88	0.70	4.3	1.8
34	30.0	2.8	0.50	1.9	9.5	0.6	5.0	91	0.54	4.4	1.7
35	22.0	6.2	0.12	2.5	12.5	0.5	5.0	92	0.28	2.7	1.9
25	65.0	65.0	0.08	2.5	12.5	1.0	3.0	85	0.04	9.1	2.6

[a]At 30 °C, 15h.
[b]Ammonium peroxodisulfate.
[c]*N,N,N′,N′*-Tetramethylethylenediamine.
[d]Calculated by SEC, standard pullulans, in PBS.

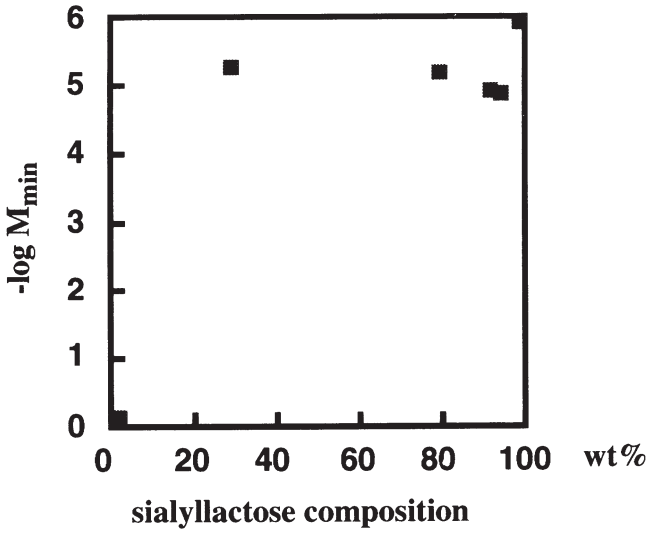


Figure 1. Dependence of copolymer compositions on hemagglutination inhibition. M_{min}: a minimum molar concentration to inhibit hemagglutination.

tact inhibition of virions by the polymer was interpreted in terms of steric stabilization by Whitesides *et al.* [19–22].

Neutralization of influenza viruses by glycopolymers

The binding of glycopolymers to influenza viruses was examined as their cytopathic effect on MDCK (Madine-Darby canine kidney) cells. After influenza viruses had been inoculated onto monolayers of MDCK cells, the viral-induced injury to the cells was observed under a light microscope. However, injured cells were not detected when the inoculation was carried out after treatment of the MDCK cells with an appropriate amounts of the homopolymer, copolymers, and naturally-occurring fetuin. It was thus confirmed that the cells were not affected by these glycopolymers. The glycopolymers caused the neutralization of influenza viruses.

The neutralization was assessed as the decrease in the amount of lactate dehydrogenase (LDH) released from the infected cells [34,35]. Figure 2 illustrates the protcol. Increasing concentrations of the glycopolymers were added to monolayers of MDCK cells, influenza viruses were inoculated, and then the amount of LDH released was determined. The decrease in LDH activity relative to the controls in the absence of the glycopolymers was estimated as a measure of the neutralization.

Figure 3 plots the decrease in LDH activity as a function of the concentrations of the sialyllactose-carrying homopolymer, copolymers, and fetuin. The homopolymer induced the neutralization of influenza infection by the A/PR/8/34 strain at concentrations higher than 1 μg/mL, the median effective concentration (EC_{50}) being ~10 μg/mL. Copolymer samples with four different compositions showed al-

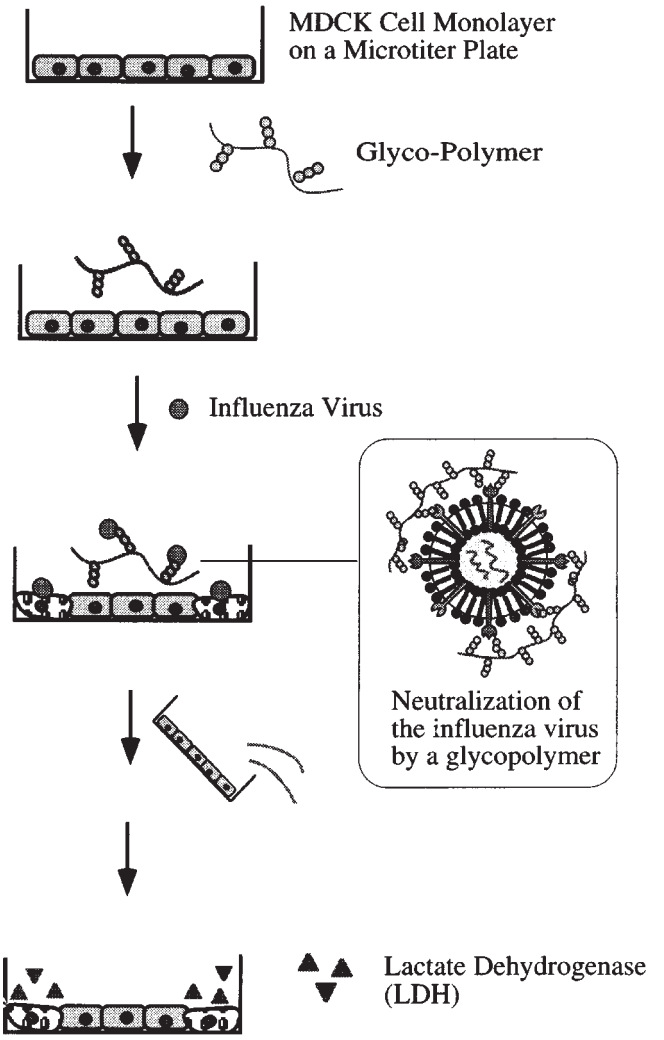


Figure 2. The inhibition of the virus-induced cytopathic effect by the glycopolymer: Colorimetric assay of LDH activity on a monolayer of MDCK cells on a microtitre plate.

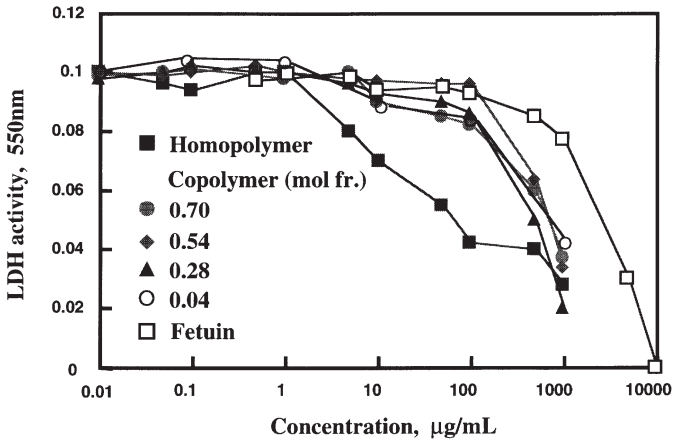


Figure 3. Inhibition of the cytopathic effect (CPE) of influenza virus A/PR/8/34 by glycopolymers.

most the same effect, and their EC_{50}s were ~5×10^2 μg/mL. This tendency was the same as that of EC_{50} based on the molar concentration of the sialyllactose moiety. Fetuin was required at a concentration higher than 100 μg/ml, and its EC_{50} was ~5×10^3 μg/mL. The corresponding lactose-carrying homopolymer exhibited no inhibitory activity (the data not shown). The sialyllactose-carrying homopolymer was about 100 times more effective than fetuin and the copolymers were about 10 times more effective than fetuin.

The inhibition by the homopolymer was also investigated using viruses with different linkage specificities and is compared with that of glycophorin in Figure 4. Strain A/PR/8/34 was neutralized by the homopolymer much more effectively than by glycophorin. Since the A/PR/8/34 strain is specific to α(2-3) rather than α(2-6)-linked sialyllactose, the present polymer having a larger amount of α(2-3) linked sialyllactose induced the neutralization more effectively. Strain A/Memphis/1/71 was slightly more affected by the homopolymer than by glycophorin. Strain B/Lee/40 was bound to neither the homopolymer nor to glycophorin. These strains exhibit α(2-6) specificity rather than α(2-3) specificity, and hence the effect of the homopolymer was decreased. The inhibitory activity of the homopolymer was found to be related to the linkage specificity of the virus strains.

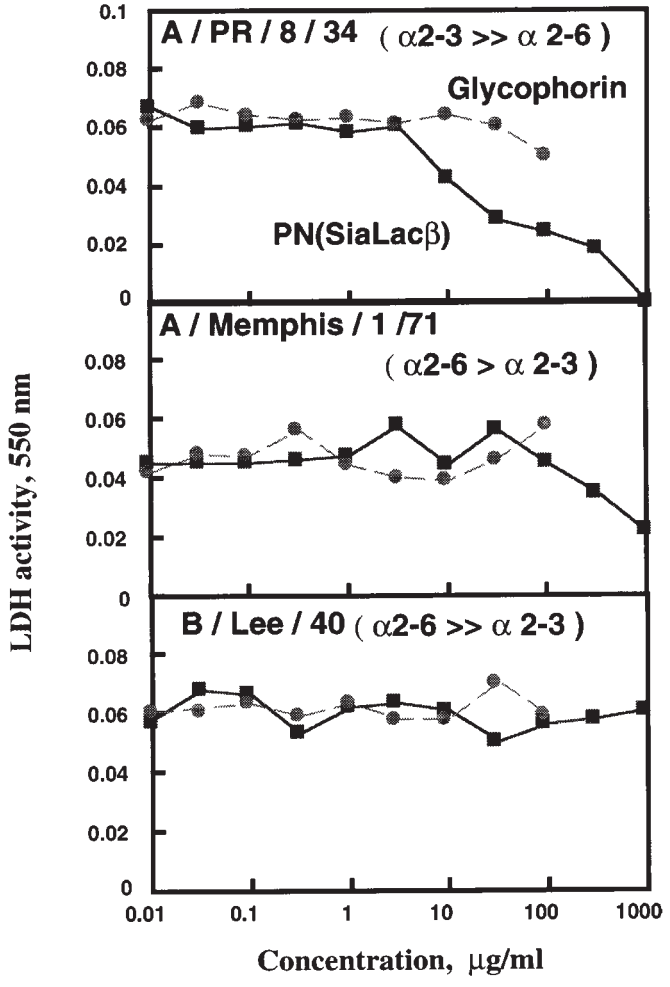


Figure 4. Inhibition of the cytopathic effect (CPE) of various influenza virus strains (A/PR/8/34, A/Memphis/1/71, and B/Lee/40) by glycopolymers.

Trapping of influenza viruses by glycopolymers immobilized on microtiter plates

One of the most important characteristics of the present glycopolystyrenes is their strong adsorption ability as to hydrophobic surfaces due to their amphiphilic properties [39,40]. The adsorption ability of the present glycopolymer has been applied for trap influenza viruses. As shown in Figure 5, the glycopolymer was immobilized on the surface

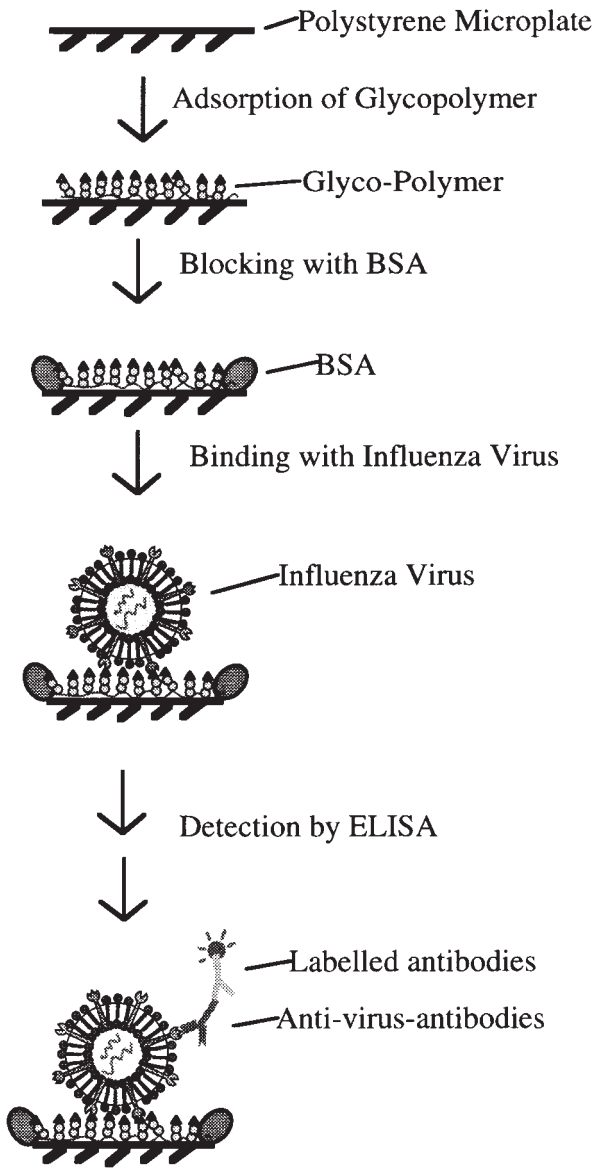


Figure 5. The ELISA assaying of influenza viruses trapped by the immobilized glycopolymer on a microtiter plate.

of microtiter plates and then viruses were added, followed by rinsing several times. The amounts of the viruses trapped were determined by an enzyme-linked immunosorbent assay (ELISA).

Figure 6 plots the absorption in the ELISA assay as a function of the amounts of three viruses with different linkage specificities. It was found that the amounts of trapped viruses increased with increases in the concentrations of the viruses, depending on their linkage specificities. The amounts trapped increased in the order of B/Lee/40 < A/Memphis/1/71 < A/PR/8/34. The virus strains with α(2-3) linkage specificity were trapped more strongly than those with α(2-6) linkage specificity. This order was the same as the results of inhibition mentioned above.

Discussion of the interactions between the glycopolymers and influenza viruses

It was found that glycopolymers carrying a mixture of α(2-3) and α(2-6) linked sialyllactose isomers as pendant groups are potent inhibitors of influenza viruses at low concentrations. The hemagglutination inhibition and neutralization activities of the homopolymer were higher than those of naturally-occurring fetuin and glycophorin. Influenza viruses were trapped by the glycopolymers adsorbed on the surface of microtiter plates. The glycopolymers showed the interaction with influenza viruses not only in solution but also on a solid surface. The inhibitory and trapping abilities of the glycopolymers depended on the linkage specificities of the virus strains. The α(2-3) specific viruses were bound more strongly to the present glycopolymers, which carry larger amounts of the α(2-3) linked isomer than the α(2-6) linked isomer.

Whitesides *et al.* [16,19–22] reported a hemagglutination inhibition assay involving polymers carrying α-C-sialic acids under various conditions. Their glycopolymers were reported to inhibit hemagglutination 10^6 times as strongly as sialic acid does. Our present polymer showed inhibition 10^3 times as strong as that of sialyllactose. Although sialyllactose was reported to bind influenza viruses more strongly than sailic acid itself, the inhibition abilities of these two glycopolymers can not be compared directly. Nagy *et al.* [17] carried out an plaque assay using another type of α-C-sialoside glycopolymer. The number of plaques was reduced and approximately 50% inhibition of plaque formation was attained with the concentration of 100 μM of the glycopolymer. The present α-O-sialo-glycopolymer inhibited infection by the A/PR/8/34 strain by approximately 50% at the concentration of 10 μM. Although neuraminidase is known to cleave O-sialoside more easily than C- and S-sialosides, the present glycopolymers carrying α-O-sialoside showed rather strong inhibitory ability.

We assumed that (1) the binding between the present glycopolystyrene and influenza viruses was enhanced by multivalent ligands, hydrophobic aglycons, and steric stabilization, and (2) highly dense polyvalent ligands might be resistant to the degradation by neuraminidase or the inhibitory ability was maintained even after partial degradation.

Charych *et al.* [18,41] reported direct colorimetric detection of influenza viruses using artificial bilayer membranes incorporating gangliosides. More recently, a lysoganglioside/poly-L-glutamic acid conjugate has been reported to inhibit influenza hemagglutinin in a picomolar concentration [42].

One advantage of the present glycopolystyrene over other glycopolymers is the simple synthetic procedure

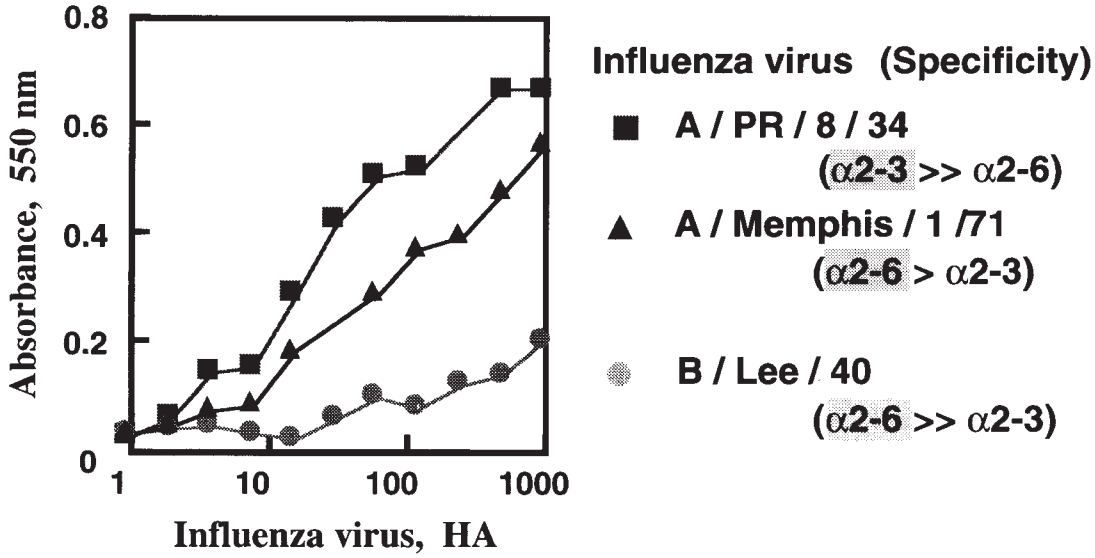


Figure 6. Trapping of influenza virus strains with various linkage specificities (A/PR/8/34, A/Memphis/1/71, and B/Lee/40) by the immobilized glycopolymer on a microtiter plate. Each well was modified with a homopolymer solution (10μg/mL).

starting from naturally occurring sialyllactose. The immobilization of the resulting polymer on hydrophobic surfaces was quite easy. We are now exploring applications of the glycopolystyrene to molecular biology and biotechnology using sialyllactose-mediated biosignaling.

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