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The hemagglutinins of the human influenza viruses A and B recognize different receptor microdomains

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A cryptically I-active sialylglycoprotein (glycoprotein 2) isolated from bovine erythrocyte membranes as Sendai virus receptor (Suzuki, Y., Suzuki, T. and Matsumoto, M. (1983) J. Biochem. 93, 1621–1633) contains *N*-glycolylneuraminic acid (NeuGc) as its predominate sialic acid and exhibits poor receptor activity for a variety of influenza viruses. Enzymatic modification of asialoglycoprotein-2 to contain *N*-acetylneuraminic acid (NeuAc) in the NeuAcα2–3Gal and NeuAcα2–6Gal sequences using specific sialyltransferase resulted in the appearance of receptor activity toward human influenza viruses A and B. The biological responsiveness chicken erythrocytes treated with sialidase and then reconstituted with derivatized glycoprotein 2 showed considerable recovery to influenza virus hemagglutinin-mediated agglutination, low-pH fusion and hemolysis. Specific hemagglutination inhibition activity of derivatized glycoprotein 2 was 5–16-times higher than that of human glycophorin. A/PR/8/34 (H_1N_1) virus preferentially recognized derivatized glycoprotein 2 containing NeuAcα2–3Gal sequence over that containing NeuAcα2–6Gal while the specificity of A/Aichi/2/68 (H_3N_2) for the sialyl linkages was reversed. B/Lee virus recognized both sequences almost equally. The biological responsiveness to the viruses of the erythrocytes labeled with the derivatized glycoprotein 2 containing NeuGc was considerably lower than that of derivatized glycoprotein 2 containing NeuAc. The results demonstrate that the hemagglutinins of human isolates of influenza viruses A and B differ in the recognition of microdomains (NeuAc, NeuGc) of the receptors for binding and fusion activities in viral penetration and the sequence to which sialic acid (SA) is attached (SAα2–3Gal, SAα2–6Gal). Inner I-active neolacto-series type II sugar chains may be important in revealing the receptor activity toward the hemagglutinin of both human influenza viruses A and B.

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Abbreviations: NeuAc, *N*-acetylneuraminic acid; NeuGc, *N*-glycolylneuraminic acid; Cer, ceramide; G_{M3}(NeuGc), NeuGcα2–3Galβ1–4Glcβ1–1Cer; IV^3(NeuAc)nLc4Cer, NeuAcα2–3Galβ1–4GlcNAcβ1–3Galβ1–4Glcβ1–1Cer; IV^6(NeuAc)nLc4Cer, NeuAcα2–6Galβ1–4GlcNAcβ1–3Galβ1–4Glcβ1–1Cer; i-active ganglioside, VI^3(NeuAc)-nLc6Cer, NeuAcα2–3Galβ1–4GlcNAcβ1–3Galβ1–4GlcNAcβ1–3Galβ1–4Glcβ1–1Cer; I-active ganglioside, NeuAcα2–3Galβ1–4GlcNAcβ1–3(Galα1–3Galβ1–4GlcNAcβ1–6)Galβ1–4GlcNAcβ1–3Galβ1–4Glcβ1–1Cer; GP-2, glycoprotein 2; SA, sialic acid; glycoprotein 2 erythrocytes, erythrocytes which had been treated with sialidase and then integrated with glycoprotein 2; HAU, hemagglutinating units. Gangliosides are abbreviated according to Svennerholm [1] and the recommendation of the IUPAC-IUB Commission on Biochemical Nomenclature [2]. All sugars mentioned in this paper were of D-configuration.

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Introduction

Recently, we isolated a biologically multifunctional sialylglycoprotein (glycoprotein 2) from bovine erythrocyte membranes [3]. This showed high receptor activity toward Sendai virus [3,4] and *Mycoplasma pneumoniae* [5,6] and also high cryptic I-antigen and heterophil Hanganutziu-Deicher (H-D) antigen activities [7]. However, no hemagglutination inhibition activity towards the influenza virus A/NWS (H_1N_1) was detectable [3]. Glycoprotein 2 contains unique high-molecular-weight (M_r 4000–9000) sugar chains, termed erythroglycan II [8], which possess endo-β-galactosidase-resistant, highly branched and heterogenous oligosaccharides of poly-*N*-acetyllactosamine type (type II chain) linked *O*-glycosidically to the peptide backbone through *N*-acetylgalactosamine. It also contains endo-β-galactosidase-susceptible straight polylactosaminyl side chains terminating mainly in Galβ1–4GlcNAcβ1–3Gal [8]. In contrast, human glycophorin, which had been reported to be an influenza virus receptor, contains short *O*-glycosidically-linked oligosaccharides

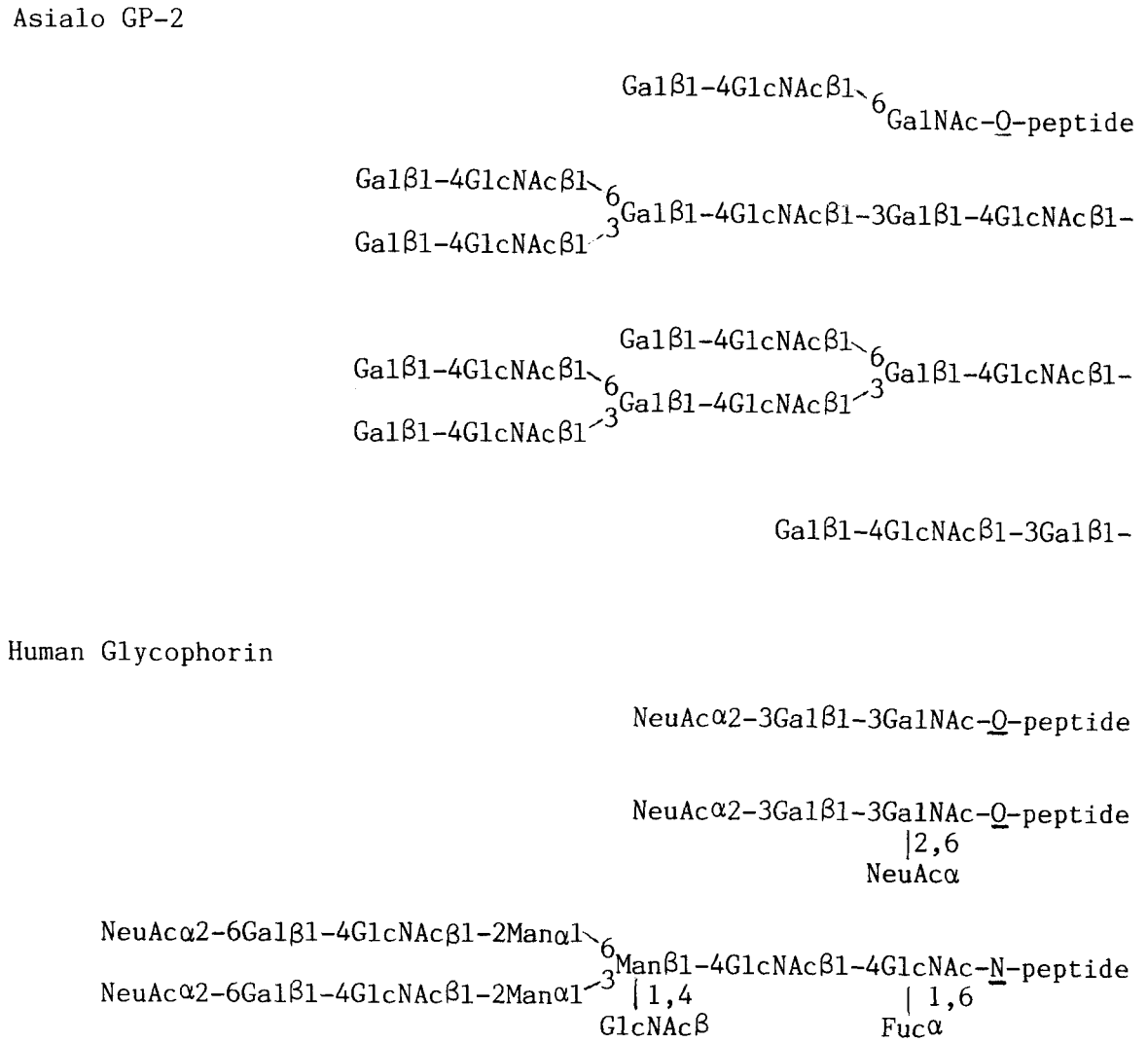

Fig. 1. Carbohydrate sequences in asialoglycoprotein 2 [5,8] and human glycophorin [9,10]. All sugar chains in asiologlycoprotein 2 link *O*-glycosidically to the peptide backbone through *N*-acetylgalactosamine.

based on the disaccharide core-region sequence, Galβ1–3GalNAc as predominate sugar chains (Fig.1) [9].

The molecular species of sialic acid in glycoprotein 2 are NeuGc (96%) and NeuAc (4%) and most of the nonreducing terminal galactose may be sialylated [8], whereas the sialic acid in human glycophorin is NeuAc [9,10]. Evidence for the presence of a NeuGcα2–3Gal sequence at the nonreducing terminus of glycoprotein 2 has been obtained from binding studies with purified anti-G_{M3}(NeuGc) antibody [7].

In this study, we developed a sensitive method for the detection of the receptor determinant of sialylglycoproteins toward influenza virus hemagglutinin. This method involves the integration of the derivatized sialylglycoprotein, which had been introduced with a single sialyloligosaccharide receptor determinant, by using specific sialyltransferases, into asialoerythrocytes, and estimation of the restoration of influenza virus hemagglutinin-mediated agglutination at 4°C, and low-pH fusion and hemolysis at 37°C. It was found that replacement of NeuGc of glycoprotein 2 with NeuAc revealed high receptor activity toward influenza viruses A and B. It was also found that the hemagglutinin of the viruses recognizes the sialic acid species (NeuAc, NeuGc) and SAα2–3Gal and SAα2–6Gal sequences of membrane-associated I-antigenically active oligosaccharides in sialylglycoprotein.

Materials and Methods

Viruses. Seed stocks of human influenza virus isolates A/PR/8/34 (H_1N_1), A/Aichi/2/68 (H_3N_2) and B/Lee/40 were generously provided by Dr. Nerome, National Institute of Health, Tokyo. These viruses were grown in 11-day-old embryonated chicken eggs and were purified by sucrose density gradient centrifugation as described previously [11].

Biological activities of influenza viruses. Hemagglutination titer, hemolysis and cell fusion activities, at low pH, were examined as described previously [12,13]. Hemagglutination titers were expressed as the highest dilution of the virus suspension resulting in complete hemagglutination of the erythrocytes.

Sialylglycoprotein glycoprotein 2 from bovine erythrocyte membranes and human glycophorin. The sialylglycoprotein glycoprotein 2, of M_r 250000, was isolated from bovine erythrocyte membranes by using lithium diiodosalicylate, phenol partitioning and ethanol precipitation, following by sequential column chromatography on Sepharose CL-4B (Pharmacia) in the presence of SDS, as described previously [3]. Human glycophorin was isolated from human erythrocytes (type 0) [14].

Preparation of derivatized glycoprotein 2. For the preparation of asialoglycoprotein 2, a reaction mixture containing glycoprotein 2 (1.0 mg) and 0.1 unit of proteinase-free sialidase (*Arthrobacter ureafaciens*, Nakarai Chemicals Ltd., (Kyoto) in 1.0 ml of 10 mM acetate buffer (pH 5.0) was incubated for 30 min at 37°C to remove sialic acid. More than 98% of the sialic acid was removed under these conditions. Asialoglycoprotein 2 was isolated by gel filtration on a Sephadex G-100 column (Pharmacia) [4]. Then sialic acid determinants were restored in a single and defined linkage by treating the asialoglycoprotein 2 with CMP[^{14}C]NeuAc (New England Nuclear, Boston) or CMP-NeuGc in the presence of highly purified sialyltransferases [15,16]. The sialyltransferases employed in this study were Galβ1–3(4)GlcNAc α2–3 sialyltransferase (EC 2.4.99.5) and Galβ1–4GlcNAcα2–6 sialyltransferase (EC 2.4.99.1) purified from rat liver [15]. The incorporation of sialic acid into glycoprotein 2 was as follows: GP-2-(NeuAcα2–3Gal), 899 nmol/mg protein; GP-2-(NeuAcα2–6Gal), 912 nmol/mg protein; GP-2-(NeuGcα2–6Gal), 566 nmol/mg protein.

Tritium labeling of the sialylglycoprotein. ^{3}H labeling of glycoprotein 2 by reductive methylation of free amino groups in the peptide backbone [17] was achieved as described previously [4].

Preparation of glycoprotein 2 or derivatized glycoprotein 2 erythrocytes: incorporation of the glycoprotein into chicken asialoerythrocytes. ^{3}H-labeled Glycoprotein 2 or ^{14}C-derivatized glycoprotein 2 (each 4 μg as sialic acid) in 0.1 ml of phosphate-buffered saline (pH 7.2) was added to 1.0 ml of 10% (v/v) asialoerythrocytes suspended in phosphate-buffered saline, and the mixture was incubaed at 37°C for 30 min. After incubation, the cells were washed three times with 4 ml of cold phosphate-buffered saline and, finally, resus-

pended in phosphate-buffered saline to obtain a 2% (v/v) cell suspension. For determination of the amount of the radioactive sialylglycoprotein in the labeled cells, 400 μl of a 2% (v/v) cell suspension, 50 μl of 30% H_2O_2 and 100 μl of Protosol (New England Nuclear) were placed in a vial. After solubilization of the cells, 1 drop of acetic acid and 5.0 ml of scintillation cocktail (Scintisol WX-H, Wako Pure Chemicals, Osaka) were added and the radioactivities were determined in a liquid scintillation spectrometer (Aloka 602).

Examination of influenza virus receptor activity of sialylglycoprotein by determination of the virus-mediated hemagglutination, low-pH fusion and hemolysis. Receptor activities of derivatized glycoprotein 2 toward influenza viruses were determined by the estimation of the restoration of hemagglutination, fusion and hemolysis of erythrocytes which had been treated with sialidase and then integrated with derivatized glycoprotein 2 [4,12]. For the estimation of the restoration of virus-mediated hemagglutination, influenza virus suspension (10 μl, 2^{10} HAU) in phosphate-buffered saline was added to 0.1 ml of sialylglycoprotein-coated erythrocytes (2%, v/v), and kept at 4°C for 5 min. Agglutination of the cells was checked by microscopic observation. For the estimation of virus-mediated cell fusion, a phosphate-buffered saline suspension of sialylglycoprotein erythrocytes (100 μl) was mixed with the virus suspended in the same buffer (10 μl, 2^{10} HAU), kept at 4°C for 5 min, washed with saline, resuspended in 20 mM acetate-buffered saline (pH 5.5 for influenza virus A/PR/8/34 (H_1N_1), pH 5.2 for A/Aichi/2/68 (H_3N_2) and pH 5.7 for B/Lee/40) and incubated at 37°C for 3 min. Cell fusion was observed by phase-contrast microscopy. For the determination of the virus-mediated hemolysis, 1.0 ml of the 2% suspension of sialylglycoprotein erythrocytes in phosphate-buffered saline was mixed with 50 μl of influenza virus (2^{10} HAU) suspended in the same buffer and kept at 4°C for 5 min. Aggregates were then washed with saline once and resuspended in 1.0 ml of 20 mM acetate-buffered saline (pH 5.5 for A/PR (H_1N_1), pH 5.2 for A/Aichi (H_3N_2) and pH 5.7 for B/Lee) and incubated with shaking at 37°C for 30 min. Hemoglobin in the supernatant obtained after low-speed centrifugation was estimated at 540 nm. The binding activity of human glycophorin and derivatized glycoproteins 2 with influenza viruses was assayed by a hemagglutination inhibition test as described previously [3].

Analytical methods. Sialic acid and protein were determined as described elsewhere (Refs. 18 and 19, respectively).

Results

Incorporation of sialylglycoprotein into asialoerythrocytes

Radioactive glycoprotein 2 containing the [^{14}C]NeuAcα2–3Gal sequence (GP-2(NeuAcα2–3–Gal)), the [^{14}C]NeuAcα2–6Gal sequence (GP-2(NeuAcα2–6Gal)) or the [^{14}C]NeuGcα2–3Gal sequence (GP-2(NeuGcα2–6Gal)) as a single receptor determinant in sialyloligosaccharides of glycoprotein 2, or native ^{3}H-glycoprotein 2, was incorporated into chicken asialo erythrocytes (amount of the sialylglycoprotein added, 5.0–5.2 pmol/10^6 cells) by incubation with the cells at 37° for 30 min. The amount of the native and derivatized glycoprotein 2 bound to the cells was about 0.1 ± 0.03 pmol sialic acid per 10^6 cells.

Generation of receptor activities toward influenza viruses by the derivatized glycoprotein 2

Agglutination of erythrocytes treated with sialidase and integrated with derivatized glycoprotein 2 (derivatized glycoprotein 2 erythrocytes) by influenza viruses. Table I demonstrated the agglutination by influenza viruses A/PR/8/34 (H_1N_1), A/Aichi/2/68 (H_3N_2) and B/Lee/40 of the chicken erythrocytes, prepared by integration of derivatized glycoprotein 2, i.e., GP-2(NeuAcα2–6Gal), GP-2(NeuAcα2–3Gal) or GP-2(NeuGcα2–6Gal), or native glycoprotein 2 containing NeuGcα2–3Gal (96%) and NeuAcα2–3Gal (4%) into the membrane surface of asialoerythrocytes. No agglutination of asialoerythrocytes or erythrocytes reconstituted with native glycoprotein 2 was observed. In the case of derivatized glycoprotein 2 erythrocytes, remarkable of agglutination was observed. Resialylation of the I-antigenically active desialylated glycoprotein 2, generated binding activity toward isolates of the human influenza virus A and B. Influenza virus A/Aichi (H_3N_2) exhibited the highest binding specificity toward

TABLE I

AGGLUTINATION OF DERIVATIZED GLYCOPROTEIN 2-COATED CHICKEN ERYTHROCYTES BY INFLUENZA VIRUSES

Chicken asialoerythrocytes were prepared as described in Materials and Methods. Reaction mixtures (1.0 ml) containing chicken asialoerythrocytes (10%, v/v) and 4 μg (as sialic acid) of derivatized glycoprotein 2 or native glycoprotein 2 were incubated at 37°C for 30 min and the cells were washed with cold phosphate-buffered saline three times and resuspended in phosphate-buffered saline. Hemagglutination titer was expressed as the highest dilution of the virus suspension giving complete agglutination of the erythrocytes.

Chicken erythrocytes resialyltated and then integrated with	Hemagglutination titer caused by influenza viruses		
	A/PR/8/34 (H_1N_1)	A/Aichi/2/68 (H_3N_2)	B/Lee/40
GP-2(NeuAcα2–6Gal) [a]	32	128	128
GP-2(NeuAcα2–3Gal) [b]	128	32	128
GP-2(NeuGcα2–6Gal) [c]	0	2	0
Native glycoprotein 2	0	0	0
Native erythrocytes	256	256	256
Asialoerythrocytes	0	0	0

[a] Glycoprotein 2 containing NeuAcα2-6Gal sequence.
[b] Glycoprotein 2 containing NeuAcα2-3Gal sequence.
[c] Glycoprotein 2 containing NeuGcα2-6Gal sequence.

terminal NeuAcα2–6Gal sequence in I-active, neolacto-series (type II) sugar chain backbone, followed by the NeuAcα2–3Gal sequence, and showed low binding specificity to the NeuGcα2–6Gal sequence, on the other hand, binding specificity of A/PR virus to the membrane-associated derivatized glycoprotein 2 was reversed. B/Lee virus recognized both GP-2(NeuAcα2–3Gal) and GP-2(NeuAcα2–6Gal) almost equally.

Inhibition of influenza virus hemagglutination by derivatized glycoprotein 2. Glycoprotein 2 containing NeuGc as its predominate sialic acid exhibited poor receptor activity toward influenza viruses A/PR/8/34 (H_1N_1), A/Aichi/2/68 (H_3N_2) and B/Lee/40. Derivatized GP-2(NeuAcα2–3Gal) and GP-2(NeuAcα2–6Gal) exhibited the highest specific inhibitory effect on the hemagglutination by A/PR and A/Aichi viruses, respectively (Table II). The inhibitory activities were 5–16-times higher than those of human glycophorin. The NeuAc-containing derivatized glycoproteins 2 inhibited equally the hemagglutination by B/Lee. The results above indicate that the inner I-active neolacto-series sugar chains in glycoprotein 2 are more critical than those in human glycophorin which had been reported to be a molecule of influenza virus receptor [14].

TABLE II

INHIBITION OF INFLUENZA VIRUS HEMAGGLUTINATION BY HUMAN GLYCOPHORIN AND DERIVATIZED GLYCOPROTEIN 2

Results are expressed as the minimum concentration of sialylglycoprotein causing complete inhibition of agglutination of native chicken erythrocytes by 4 hemagglutinating units of influenza virus, A/PR/8/34 (H_1N_1), A/Aichi/2/68 (H_3N_2) or B/Lee/40. Data represent average of three determinations.

Sialylglycoproteins	Minimum concentration (μg/ml) causing complete inhibition of hemagglutination induced by		
	A/PR/8/34 (H_1N_1)	A/Aichi/2/68 (H_3N_2)	B/Lee/40
Human glycophorin	25.5	19.5	25.5
GP-2(NeuAcα2–3Gal)	1.2	4.1	2.4
GP-2(NeuAcα2–6Gal)	4.5	1.2	2.4
Native glycoprotein 2	> 910	> 910	> 910

Low pH fusion and hemolysis of derivatized glycoprotein 2 erythrocytes by influenza viruses: recognition specificity of influenza virus hemagglutinin for sialic acid species and the sequence to which sialic acid is attached. The chicken asialoerythrocytes reconstituted with derivatizedglycoprotein 2 also exhibited recovery of influenza virus-mediated low-pH fusion and hemolysis. Fig. 2 (a,b,c,d) shows that derivatized glycoprotein 2 erythrocytes coated with GP-2(NeuAcα2–6Gal) (b) or GP-2(NeuAcα2–3Gal) (c) were effectively fused by the virus at pH 5.2 at 37°C. The erythrocytes coated with GP-2(NeuGcα2–6Gal) (d) showed low fusion activity. These results indicate that each derivatized glycoprotein 2 can function as a reporter which mediates the adsorption and fusion process of A/Aichi virus to the target cells. The activity of the influenza virus receptor mediating the adsorption and fusion process was quantified by estimation of the recovery of virus-mediated hemolysis of derivatized glycoprotein 2 erythrocytes (Fig. 3). It was found that derivatized glycoprotein 2 containing NeuAcα2–3Gal sequences

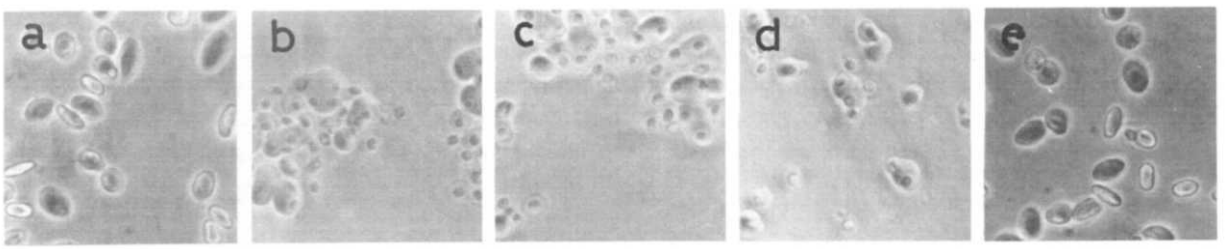

Fig. 2. Low-pH fusion of derivatized glycoprotein 2 erythrocytes by influenza virus A/Aichi/2/68 (H_3N_2). Native glycoprotein 2 erythrocytes (a), GP-2(NeuAcα2–6Gal) erythrocytes (b), GP-2(NeuAcα2–3Gal) erythrocytes (c), GP-2(NeuGcα2–6Gal) erythrocytes (d) and asialoerythrocytes (e) were incubated with influenza A/Aichi virus (2^{10} HAU) in 20 mM acetate-buffered saline (pH 5.2) at 37 °C for 3 min, and cell fusion was observed microscopically.

was the most active in terms of the restoration of hemolysis by A/PR/8/34 (H_1N_1), followed by that containing NeuAcα2–6Gal, and native glycoprotein 2. Recovery of the hemolysis of GP-2(NeuGcα2–6Gal) erythrocytes was very low. On the other hand, the most potent receptor determinant for A/Aichi virus bearing H_3 hemagglutinin was identified as the NeuAcα2–6Gal sequence, followed by NeuAcα2–3Gal, NeuGcα2–6Gal and NeuGcα2–3Gal (a major sequence in native glycoprotein 2). B/Lee virus recognized NeuAcα2–3Gal and NeuAcα2–6Gal sequences almost equally.

Further treatment of the reconstituted erythrocytes with sialidase resulted in the complete abolition of the response to influenza viruses, indicating that the exogenous sialylglycoprotein glycoprotein 2 and its derivatives could be functionally integrated into the surface membranes of asialoerythrocytes, extending the sialyl sugar chains our of the lipid bilayer of the erythrocytes, and that the introduction of a single new sialylo-

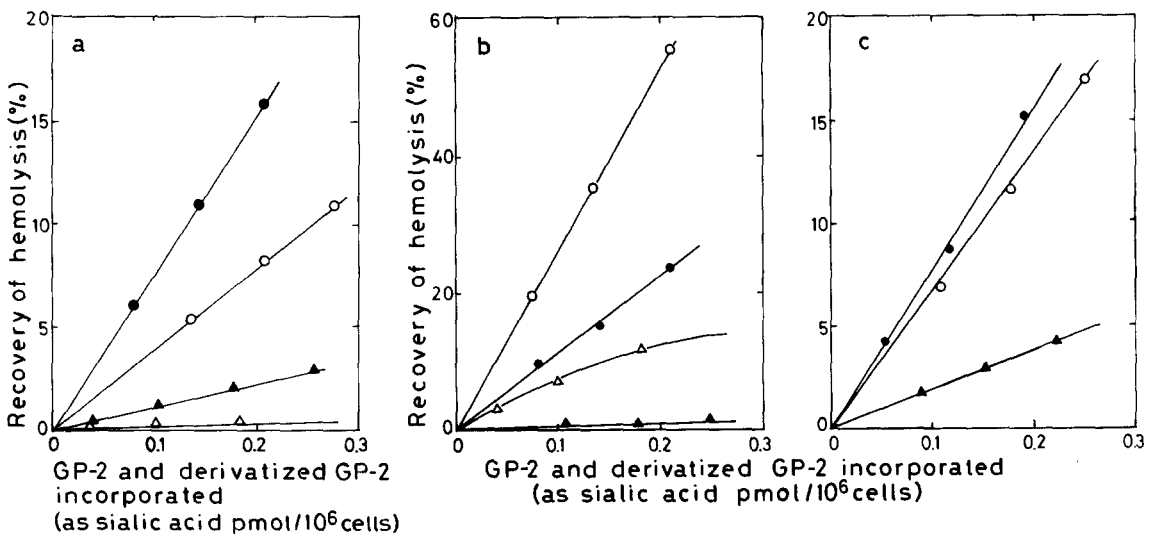

Fig. 3. Influenza virus-mediated low-pH hemolysis of chicken asialoerythrocytes reconstituted with derivatized glycoprotein 2 containing NeuAcα2–3Gal, NeuAcα2–6Gal or NeuGcα2–6Gal as a single receptor determinant in the sialyloligosaccharides of glycoprotein 2 glycoprotein. Radioactive derivatized glycoprotein 2 erythrocytes were prepared and the radioactivities were determined as described in Materials and Methods. GP-2(NeuAcα2–3Gal) (●), GP-2(NeuAcα2–6Gal) (○), or GP-2(NeuGcα2–6Gal) (△) erythrocytes or native glycoprotein 2 erythrocytes (▲) were mixed with 2^{10} HAU of influenza virus A/PR/8/34 (H_1N_1) (a), A/Aichi/2/68 (H_3N_2) (b) or B/Lee (c) and kept at 4 °C for 5 min in phosphate-buffered saline (pH 7.2), then the cells were washed with saline and resuspended in 1.0 ml of 20 mM acetate buffered saline (pH 5.5 for A/PR, pH 5.2 for A/Aichi, and pH 5.7 for B/Lee). Virus-mediated hemolysis was determined as described in the text.

ligosaccharide sequence into glycoprotein 2 could generate receptor activity toward influenza viruses.

Discussion

Glycoprotein 2, the sialylglycoprotein of bovine erythrocyte membranes possesses I-active branched, *O*-glycosidically linked sugar chains of the neolacto series poly-*N*-acetyllactosamine type (type II chains), and also contains straight terminal polylactosaminyl side chains containing the NeuGcα2–3Galβ1–4GlcNAcβ1–3Gal sequence [5,6,8]. On the other hand, human glycophorin contains 15 *O*-glycosidically linked chains with short Galβ1–4GalNAc sequences [9] and one biantennary complex carbohydrate chain, linked *N*-glycosidically to asparagine [10]. The results of the present study indicated that the sugar chains of glycoprotein 2, function as a common sugar spacer between terminal sialic acids and the peptide backbone of the receptor for the hemagglutinin of H_1 and H_3 sero-types of human influenza A viruses and B/Lee virus, and the core structure with neolacto-series poly-*N*-acetyllactosamine type in glycoprotein 2 may be more important as a functional receptor of influenza viruses than that with the short disaccharide, Galβ1–4GalNAc–*O*-peptide sequence in human glycophorin (Fig. 1, Table II). It was also found that human isolates, A/PR/8/34 (H_1N_1) and A/Aichi/2/68 (H_3N_2), preferentially bind the sequences NeuAcα2–3Gal and NeuAcα2–6Gal, respectively, in 'modified glycoprotein 2' which had been treated with sialidase and then resialylated with specific sialyltransferases.

Recently, we showed that gangliosides containing branched and linear neolacto-series (type II chain) oligosaccharides such as NeuAc-containing I-active ganglioside, i-active ganglioside (VI^3-(NeuAc)nLc6Cer) and sialylparaglobosides (IV^3-(NeuAc)nLc4Cer and IV^6(NeuAc)nLc4Cer) were commonly recognized as influenza A virus receptors mediating the adsorption and fusion processes of the virus infection [20,21]: human influenza A viruses bearing H_1 hemagglutinin (A/PR/8/34 (H_1N_1)) exhibited preferential binding to the terminal NeuAcα2–3Gal sequence of IV^3(NeuAc)nLc4Cer, while human H3 (A/Aichi/2/68 (H_3N_3)) hemagglutinin bound preferentially to NeuAcα2–6Gal in IV^6(NeuAc)-nLc4Cer containing the same oligosaccharide backbone (neolactoseries, type II chain) as IV^3(NeuAc)nLc4Cer. The above results are consistent with those of the present studies. Rogers and Paulson [22,23] and Higa and Paulson [16] reported that influenza virus hemagglutinin binds human erythrocytes modified enzymatically to restore cell surface sialyloligosaccharides with different sialic acid species and a terminal SA–Gal sequences. The improved method developed in this study to determine functional structure of sugar chains for influenza virus receptor is based on the enzymatic modification of the sialyl residue and the SA–Gal sequence of the 'glycoprotein' by specific sialyltransferase, integration of the resialylated glycoprotein into the asialoerythrocytes, and the determination of the recovery of the virus-mediated hemagglutination, low-pH fusion and hemolysis. The merit of this method is to identify the structure of terminal and internal oligosaccharides in membrane-associated sialylglycoprotein necessary not only for binding but also for internalization of the virion by the fusion process.

In conclusion, hemagglutinin of human isolates of influenza viruses A and B distinguishes terminal microdomains of the receptor, i.e., sialic acid species (NeuAc, NeuGc) and the links to which sialic acid is attached (Saα2–3Gal, SAα2–6Gal). Inner I-active neolacto-series type II sugar chains in membrane-associated glycoproteins and gangliosides may also be important as a common part of the receptor determinant toward the hemagglutinin of human influenza viruses A and B. The systems developed in this paper for the detection of the influenza virus receptor may be useful for the determination of a functional domain of membrane-associated glycoconjugates which mediates the early stage of the infection of influenza virus, i.e., adsorption to the receptor and entrance into the endosomes and/or lysosomes where the low pH value initiates release of viral genome into the cytoplasma by fusion of the viral envelope and the membranes of these intracellular organelles [24,25].

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