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N-glycan structures of a recombinant mouse soluble Fcγ receptor II

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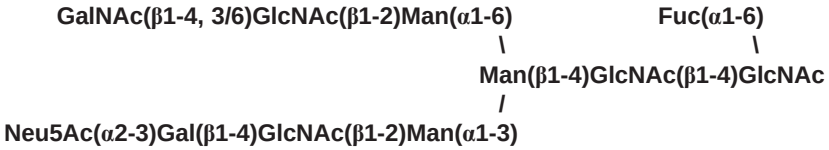
N-glycans of a recombinant mouse soluble Fcγ receptor II (sFcγRII) expressed in baby hamster kidney cells were released from glycopeptides by digestion with glycoamidase A (from sweet almond), and the reducing ends of the oligosaccharides were reductively aminated with 2-aminopyridine. The derivatized *N*-glycans were separated and structurally identified by a three-dimensional high-performance liquid chromatography (HPLC) mapping technique on three kinds of HPLC columns [Takahashi, et al. (1995) *Anal. Biochem.* 226: 139–46]. Eighteen different major *N*-glycan structures were identified, of which six were neutral (45%), five mono-sialyl (49%), one di-sialyl (4.6%), five tri-sialyl (1.1%), and one tetra-sialyl (0.3%). All *N*-glycan structures determined were complex type with fucosylation at the *N*-acetylglucosamine residue of the reducing end, and *N*-acetylneuraminic acid, when present, was α-(2,3)-linked. The existence of a unique structure containing both *N*-acetylgalactosamine and α-(2,3)-*N*-acetylneuraminic acid residues at the reducing ends, as below, was confirmed by MALDI-TOF mass spectrometry.



Keywords: **soluble Fcγ receptor II, N-glycans, 3-D sugar mapping technique**

Abbreviations: **2/3-D, two/three-dimensional; BHK, baby hamster kidney; Fuc, L-fucose; GalNAc, *N*-acetylgalactosamine; GLcNAc, *N*-acetylglucosamine; Hex, hexose; HexNAc, *N*-acetylhexoxamine; Neu5Ac or NeuAc, 5-*N*-acetylneuraminic acid; HPAEC-PAD, high-pH anion exchange chromatography with pulsed amperometric detection; HPLC, high-performance liquid chromatography; MALDI-TOFMS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; ODS, octadecyl-silica; PA, pyridylamino; PSD, postsource decay, sFcγR, soluble receptor for the Fc portion of IgG.**

Introduction

Receptors for the Fc portion of IgG (FcγRs) are a heterogeneous family of transmembrane glycoproteins that mediate a variety of effector and regulatory functions in hematopoietic cells.

In mice and humans, FcγRs have been classified into three basic types: FcγRI, FcγRII, and FcγIII [1, 2]. FcγRI has a high affinity for monomeric immunoglobulin G (IgG), while FcγRII and FcγRIII are low-affinity receptors that bind to IgG-containing immune complexes. FcγRII and FcγRIII possess similar extracellular regions composed of two Ig-like domains. A variety of isoforms of FcγR, resulting from the translation of alternatively spliced messenger ribonucleic acid (mRNAs), have been identified [3]. Some of those isoforms are expressed as soluble forms that lack the transmembrane region. Soluble forms of FcγRs (sFcγRs), consisting of their extracellular domains, have also been shown to arise from membrane FcγRs by proteolytic cleavage that occurs at the extracellular region, close to the cell surface [4].

Soluble FcγRs play important roles in regulation of immune functions, by blocking Fcγ-mediated functions through interaction with the Fc portions of IgG [5], and by binding to cell surface molecules such as complement re-

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ceptor 3 (CR3) [6]. Several lines of evidence show that glycosylation is involved in interaction of FcγRs with these two kinds of ligands. To clarify the functional roles of sFcγRs *in vitro* and to facilitate their potential use *in vivo* for treatment of immune disorders, expression systems to produce high amounts of recombinant sFcγRs have been successfully established by use of eukaryotic cell lines [7–11]. Although these recombinant sFcγRs appear to be secreted as glycoproteins [3, 7–11], little is known regarding glycosylation of sFcγR.

Determination of glycoforms is important for better use of recombinant sFcγRs as immunochemical and therapeutical reagents, as well as for furthering our understanding of their immunological roles. It is possible that specific glycoforms are associated with specific functions of sFcγR. Edberg and Kimberly [12] reported that FcγRIIIa expressed on natural killer cells and on monocytes are differentially glycosylated, with a consequent change in ligand-binding properties. *In vivo* studies indicate that glycoforms may be determining factors of sFcγR antigenicity and serum half-life. Furthermore, knowledge concerning glycoforms facilitates structural analyses of sFcγR at atomic resolution. Microheterogeneity of glycans often hampers crystallization of glycoproteins. Once chemical structures of glycans are available, the information will be useful for preparation of sFcγR with homogeneous glycans trimmed by appropriate glycosidase treatments. Such techniques could yield good crystals in the quantity required for x-ray analysis. Information on glycoforms is also useful for nuclear magnetic resonance (NMR) spectral analysis of glycoproteins.

Here we report the structures of major *N*-linked oligosaccharides from a recombinant sFcγRII expressed in baby hamster kidney (BHK) cells. These structures were successfully identified by a three-dimensional (3-D) high-performance liquid chromatography (HPLC) mapping technique on three kinds of HPLC columns.

Materials and methods

Enzymes

Glycoamidase A (glycopeptidase A) from sweet almond [13, 14], β-galactosidase, and *N*-acetylhexosaminidase from jack bean were purchased from Seikagaku Kogyo (Tokyo, Japan). Alpha-*L*-fucosidase from bovine kidney was from Boehringer Mannheim Biochemicals (Mannheim, Germany). Cloned α-(2,3)-specific sialidase from *Salmonella typhimurium* LT2 was from Takara Shuzo (Otsu, Japan). Trypsin and chymotrypsin were from Sigma (St. Louis, MO, U.S.A).

Reference oligosaccharides

Pyridylamino (PA) derivatives of isomalto-oligosaccharides (4–20 glucose residues) and of reference oligosaccharides (Code Nos 010.1, 110.1, 110.3, 210.3, and 210.4) were from Seikagaku Kogyo. The following PA-oligosaccharides were prepared by known methods: 210.4b from human urinary kallikrein [15], 1A3-210.4 from human serum [16], and 1A4-210.4 from bovine thyroglobulin. 1A2-210.3 was obtained by β-galactosidase digestion of 1A3-210.4, and 1A2-110.3 was obtained similarly by β-galactosidase and β-*N*-acetylhexosaminidase digestion of 1A4-210.4. Other mono-, di-, tri-, and tetra-sialyl oligosaccharides used as reference compounds in this article were all obtained from human integrin [17].

Other chemicals

Sephadex G-15 was from Pharmacia Biotech (Uppsala, Sweden). Sodium cyanoborohydride was from Aldrich (Milwaukee, WI, U.S.A.). 2-Aminopyridine was from Wako Pure Chemical (Osaka, Japan).

Preparation of sFcγRII

A recombinant sFcγRII with 174 amino acids was expressed in cell line 6/9CII and purified by ion exchange chromatography followed by affinity chromatography on rabbit IgG-coupled sepharose as described previously [7, 18]. Sodium dodecyl sulfate (SDS)-Polyacrylamide gel electrophoresis (PAGE) analysis showed that the material was 99% pure and migrated with apparent molecular mass 28–35 kDa before and 19 kDa after deglycosylation [18].

Preparation and derivatization of the *N*-linked oligosaccharide moiety

Three mg of the recombinant sFcγRII glycoprotein was digested with a mixture of trypsin and chymotrypsin. The peptide mixture from about 350 nmol of FcγR glycopeptides was then digested with 1 mU of glycoamidase A in 120 μl of 0.1 M citrate-phosphate buffer pH 5.0 at 37°C overnight. The resulting mixture was digested with pronase to facilitate separation of oligosaccharides from peptidic materials. The oligosaccharide mixture was applied on a Bio-Gel P-4 column (1.0 × 40 cm) and dried thoroughly. After reductive amination with 2-aminopyridine using sodium cyanoborohydride [19], PA-oligosaccharides were purified by gel filtration on a Sephadex G-15 column (1.0 × 40 cm).

Glucose unit (Glc unit)

We calibrated both octadecyl-silica (ODS) and amide-silica columns with PA-derivatized isomalto-oligosaccharide mixtures. Numbers 4, 5, 6, and so on indicate the degree of polymerization of glucose. Sample PA-oligosaccharide was applied to each column, and elution times were compared with those of the isomalto-oligosaccharide mixtures. Elution times of the sample on both columns are thus expressed in terms of glucose equivalents ("Glc units").

Isolation and characterization of each PA-oligosaccharide by 3-D mapping technique

The PA-oligosaccharide mixture was separated on an anion exchange column, TSKgel DEAE-5PW (7.5 × 75 mm, TOSOH, Japan), according to the number of sialic acid residues in each molecule. Each PA-oligosaccharide fraction eluted was then applied separately to a reversed-phase column (Shim-pack CLC-ODS, 6 × 150 mm, Shimadzu, Japan). The elution time for each peak was recorded, expressed in Glc units and plotted on the X-axis. Each oligosaccharide fraction separated on the ODS column was collected separately and applied to an amide-adsorption column (Amide-80, 4.6 × 250 mm, TOSOH). Elution conditions of the three columns were as described previously [16]. The elution time for each peak was recorded in Glc units and plotted on the Y-axis. After plotting the X and Y coordinates from all PA-oligosaccharides on a two-dimensional (2-D) map, we compared the coordinate of a given sample with those of reported standard PA-oligosaccharides. Using a computer search, we chose a few PA-oligosaccharides whose coordinates coincided with that of the given sample within allowable error (±5%). The sample PA-oligosaccharide and a standard were coinjected onto ODS and amide columns and confirmed to give a single peak. The sample and standard were also digested simultaneously with several glycosidases and their coordinates were again compared on the map. In these three HPLCs, using the LC-10A HPLC system (Shimadzu, Japan), PA-oligosaccharides were detected by fluorescence using excitation and emission at 320 and 400 nm, respectively.

Exoglycosidase digestion procedure

Each PA-oligosaccharide (50 pmol) isolated from the three successive columns was digested with exoglycosidases (sialidase, β-galactosidase, β-*N*-acetylhexosaminidase, α-*L*-fucosidase) under conditions described previously [14, 17]. The elution coordinate of each exoglycosidase-trimmed oligosaccharide was examined on the 2-D map to verify its structural identity. In the cases of oligosaccharides N-e and M-d, the *N*-acetylhexosamine released by direct β-*N*-acetylhexosaminidase digestion was determined by high-pH anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) as described previously [15].

MALDI-TOFMS analysis

The relative molecular mass of each PA-oligosaccharide was determined by PSD MALDI-TOF mass spectrometry using a Voyager Elite mass spectrometer equipped with a delayed-extraction system (PerSeptive Biosystems, Framingham, MA, U.S.A.). The sample was ionized by a nitrogen laser at 337 nm. 2,5-Dihydroxybenzoic acid was dissolved in 50% aqueous acetonitrile solution containing 0.1% trifluoroacetic acid at a concentration of 20 mg/ml. The sample (10 pmol/μl) was dissolved in distilled water and mixed with the matrix solution at a 1:9 volume ratio. One μl of aliquot was placed on the sample plate and air dried. Data were analyzed using GRAMS/386 software.

Results and discussion

Separation of oligosaccharides by three successive HPLC steps

N-glycans of recombinant sFcγRII released from the glycoprotein by glycoamidase A were derivatized with 2-aminopyridine. The PA-oligosaccharides were separated by DEAE-5PW column (first HPLC) into five fractions: neutral, mono-, di-, tri-, and tetra-sialyl oligosaccharides (Figure 1A). Molar ratio of these five fractions was 45.0, 49.0, 4.6, 1.1, and 0.3%, respectively. Although glycoamidase A is able to release sialic acid-, phosphoric acid-, sulfuric acid-, and uronic acid–containing *N*-glycans from glycopeptides, all ionic charges in the FcγR came from α-(2,3)-sialic acids, since all acidic fractions changed to neutral ones after α-(2,3)-specific sialidase (from *S. typhimurium*) digestion.

PA-oligosaccharide fractions separated on DEAE-5PW column were applied to the ODS column (second HPLC) (Figure 1B); then their individual peaks were applied to the amide column (third HPLC) (data not shown). Although neutral fraction N-c was separated into two fractions (N-c1 and N-c2) on amide column, mono-sialyl fractions M-c1 and M-c2, and tri-sialyl T-c1 and T-c2, still could not be resolved by this step. They were differentiated only after glycosidase digestion, as described below.

Structural characterization of PA-oligosaccharides using 2-D mapping technique

Structural assignment of all neutral and sialyl oligosaccharides from FcγR was performed by 2-D mapping technique, as described previously [20]. Coordinates of almost all *N*-glycans from sFcγRII coincided (± 5%) with those of known oligosaccharides on the map. Neutral PA-oligosaccharides N-a, N-b, N-c1, N-c2, N-d, and N-e, mono-sialyl oligosaccharides M-a and M-b, di-sialyl oligosaccharide D-a, tri-sialyl oligosaccharides T-a, T-b, and T-d, and tetra-sialyl oligosaccharide Te-a were assigned code numbers 010.1, 110.1, 110.3, 210.3, 210.4, and 210.4b (for neutral oligosaccharides), 1A2-210.3 and 1A2-110.3 (for mono-sialyl), 2A4-210.4 (for di-sialyl), 3A2-310.18, 3A3-410.16, and 3A4-310.8 (for tri-sialyl), and 4A2-410.16 (for tetra-sialyl), respectively (Table 1). Co-chromatography on ODS and amide columns of each of the sample PA-oligosaccharides with the corresponding reference compound confirmed these assignments.

The structural candidate for N-e chosen by a computer search was code No. 210.4b, which has two GalNAc(β1-4)GlcNAc sequences. Since presence of GalNAc is unusual among the other *N*-glycan structures in sFcγII, we confirmed the existence of GalNAc residues after β-*N*-acetyl-

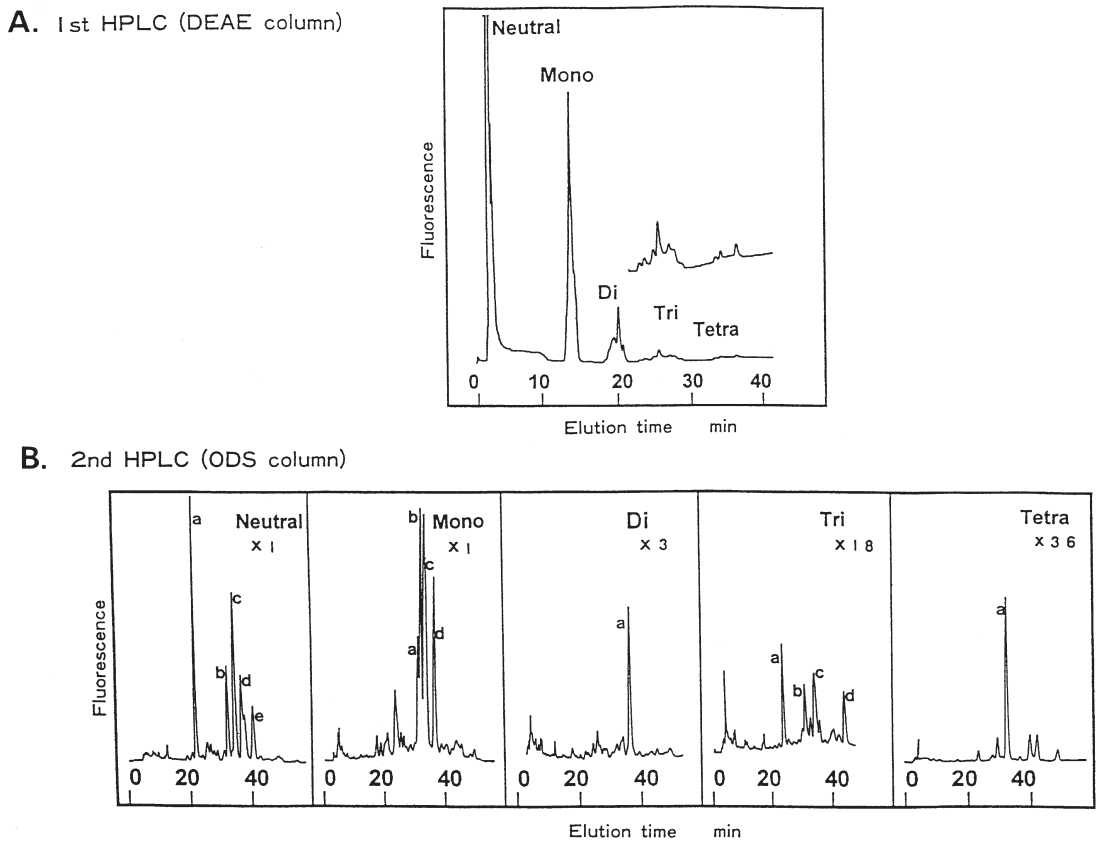
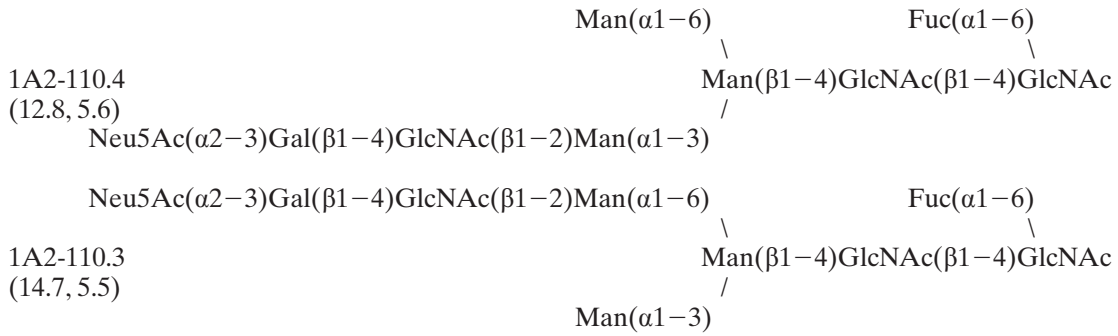


Figure 1. Pyridylamino oligosaccharide separation leading to the 3-D map. The neutral and sialylated pyridylamino oligosaccharide mixture obtained from recombinant mouse sFcγRII was separated on three different columns. **A**, by the first HPLC on the DEAE column, pyridylamino oligosaccharides are separated according to their sialic acid content. **B**, each of the neutral and mono-, di-, tri-, and tetra-sialyl oligosaccharides was individually separated on the second ODS column.

hexosaminidase digestion as described previously [15], prior to co-chromatography procedures.

Identification of M-c1 and M-c2

Mono-sialyl oligosaccharide M-c could not be separated by the above procedure. M-c was therefore digested by α-(2,3)-specific sialidase, resulting in a neutral oligosaccharide with Glc unit 13.8 on ODS column and 7.3 on amide column. This coordinate coincided with that of reference oligosaccharide code No. 210.4 (13.8, 7.3) [21] and also with that of fraction N-d from sFcγRII. Since oligosaccharide 210.4 has two Gal residues, the problem was to determine which was attached by a sialic acid. Sequential digestion of M-c with β-galactosidase and β-*N*-acetylhexosaminidase yielded the following two compounds roughly equal quantities.



We concluded that the original M-c was a 1:1 mixture of M-c1 (1A3-210.4) and M-c2 (1A4-210.4), as shown in Table 1.

Table 1. The proposed structures and the relative quantities of PA-derivatized *N*-glycans from recombinant mouse soluble FcγRII expressed in BHK cells.

Peak name code no.	*N-glycan structures*	*Glc unit ODS Amide-80*	*Yield (%)*
Neutral (6)			
	Man(α1-6)		
N-a	\		
010.1	R	10.2	13.8
	/	4.6	
	Man(α1-3)		
	GlcNAc(β1-2)Man(α1-6)		
N-b	\		
110.1	R	12.6	6.3
	/	5.1	
	Man(α1-3)		
	Gal(β1-4)GlcNAc(β1-2)Man(α1-6)		
N-c1	\		
110.3	R	13.2	11.8
	/	6.0	
	Man(α1-3)		
	GlcNAc(β1-2)Man(α1-6)		
N-c2	\		
210.3	R	13.2	1.3
	/	6.4	
	Gal(β1-4)GlcNAc(β1-2)Man(α1-3)		
	Gal(β1-4)GlcNAc(β1-2)Man(α1-6)		
N-d	\		
210.4	R	13.8	6.8
	/	7.3	
	Gal(β1-4)GlcNAc(β1-2)Man(α1-3)		
	GalNAc(β1-4)GlcNAc(β1-2)Man(α1-6)		
N-e	\		
210.4b	R	14.8	5.0
	/	6.7	
	GalNAc(β1-4)GlcNAc(β1-2)Man(α1-3)		
Mono-sialyl (5)			
	GlcNAc(β1-2)Man(α1-6)		
M-a	\		
1A2-210.3	R	14.3	6.3
	/	5.9	
	Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-2)Man(α1-3)		
	Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-2)Man(α1-6)		
M-b	\		
1A2-110.3	R	14.7	12.7
	/	5.6	
	Man(α1-3)		
	Gal(β1-4)GlcNAc(β1-2)Man(α1-6)		
M-cl	\		
1A3-210.4	R	15.2	10.0
	/	6.8	
	Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-2)Man(α1-3)		
	Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-2)Man(α1-6)		
M-c2	\		
1A4-210.4	R	15.2	10.0
	/	6.8	
	Gal(β1-4)GlcNAc(β1-2)Man(α1-3)		

Table 1. (continued)

Peak name code no.	*N-glycan structures*	*Glc unit ODS Amide-80*	*Yield (%)*
M-d 1A2-210.4a1	GalNAc(β1-4, or 3/6)GlcNAc(β1-2)Man(α1-6) \ R / Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-2)Man(α1-3)	16.3 6.6	10.0
Di-sialyl (1)			
D-a 2A4-210.4	Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-2)Man(α1-6) \ R / Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-2)Man(α1-3)	16.5 6.6	4.6
Tri-sialyl (5)			
T-a 3A2-310.18	Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-6) \ Man(α1-6) / \ Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-2) R / Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-2) Man(α1-3)	12.4 7.7	0.3
T-b 3A3-410.16	Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-6) \ Man(α1-6) / \ Gal(β1-4)GlcNAc(β1-2) R Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-4)	14.9 8.9	0.2
T-c1 3A5-410.16	\ / Man(α1-3) / Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-2) Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-6) \ Man(α1-6) / \ Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-2) R Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-4)	16.1 8.7	0.1
T-c2 3A6-410.16	\ / Man(α1-3) / Gal(β1-4)GlcNAc(β1-2) Gal(β1-4)GlcNAc(β1-6) \ Man(α1-6) / \ Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-2) R Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-4)	16.1 8.7	0.3
	\ / Man(α1-3) / Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-2)		

Table 1. (continued)

Peak name code no.	*N-glycan structures*	*Glc unit ODS Amide-80*	*Yield (%)*
T-d	Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-2) Man(α1-6) \		
3A4-310.8	R	20.4 7.4	0.2
	Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-4) \ / Man(α1-3) / Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-2)		
Tetra-sialyl (1)			
	Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-6) \ Man(α1-6) / \		
Te-a	Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-2)		
4A2-410.16	R	14.8 8.6	0.3
	Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-4) \ / Man(α1-3) / Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-2		
	Fuc(α1-6) \ R = Man(β1-4)GlcNAc(β1-4)GlcNAc		

Identification of M-d

Fraction M-d was eluted on a 2-D map at 16.3, 6.6 (Figure 2). This coordinate did not coincide with any reference coordinate on the map. The coordinates of defucosyl M-d (12.1, 6.3), desialyl M-d (15.5, 6.9), and desialyl-defucosyl M-d (11.3, 6.5) also did not coincide with any reference coordinate. Although the coordinate of M-d was not changed by β-galactosidase digestion, it changed greatly after β-*N*-acetylhexosaminidase digestion, and the resulting coordinate matched that of reference structure 1A2-110.4 (12.8, 5.6) on the 2-D map (see structure in preceding section). The decrease of Glc units on amide column from M-d (6.6) to de-*N*-acetylhexosaminyl M-d (5.6) was 1.0, corresponding to a loss of two *N*-acetylhexosamine residues [22]. Indeed, release of one mole each of GalNAc and GlcNAc residue was detected simultaneously on HPAEC-PAD column [15]. Furthermore, the presence of unsubstituted GalNAc residue at the nonreducing end of M-d was demonstrated by changing the enzyme quantities added or the incubation times of β-*N*-acetylhexosaminidase reaction mixtures (data not shown).

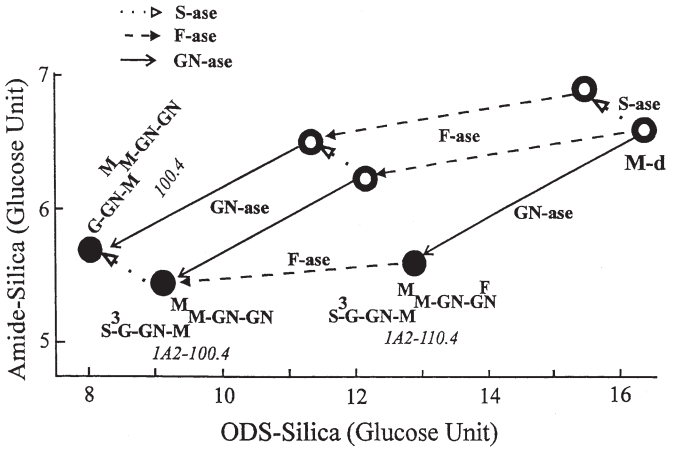
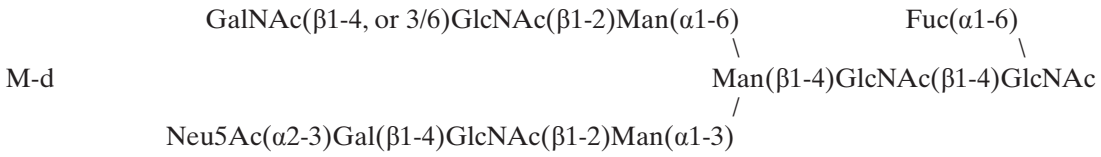


Figure 2. The change of coordinates on the 2-D map of PA-oligosaccharide M-d. Starting material M-d was sequentially digested with α-(2,3)-sialidase (⋯▷), α-fucosidase (- - -▶), and β-*N*-acetylhexosaminidase (⟶)○, unknown oligosaccharides; ●, known oligosaccharides.

Following sequential α-fucosidase and α-(2,3)-sialidase digestion of de-*N*-acetylhexosaminyl M-d (1A2-110.4), the resulting coordinate coincided with that of 100.4 (Figure 2).

MALDI-TOF MS data (Figure 3) are consistent with above results. The value of 2198.8 matches the total mass of component sugars of M-d ($NeuAc_1Gal_1HexNAc_3Man_3Fuc_1GlcNAc_2$-PA). Most of the other peaks are also reasonably interpretable as fragments. In particular, the appearance of peaks at 1995.5 and 1791.9, produced from the original 2198.8 peak by sequential deletion of two HexNAc residues, suggests the existence of GalNAc-GlcNAc sequence in the nonreducing end of the upper antenna. The peak 407.2 can be interpreted as the relative molecular mass of GalNAc-GlcNAc, corresponding to 1791.9. For the lower antenna of M-d, the situation is similar. For example, peak 1541.5 is produced from the original 2198.8 by sequential deletion of NeuAc, hexose, and HexNAc, and peak 657.6 corresponds to the detached NeuAc-Gal-GlcNAc. Thus, we propose the unique structure shown below for oligosaccharide M-d.



There are three possible linkage positions between the GalNAc and GlcNAc residues: β-(1,3), β-(1,4), and β-(1,6). The most probable one is β-(1,4) linkage, because (1) oligosaccharide M-d may be related to one of the neutral oligosaccharides of sFcγRII, N-e, which contains two GalNAc(β1-4)GlcNAc sequences. Since a GalNAc transferase system operates in the cell, the sFcγRII protein must have some other GalNAc(β1-4)GlcNAc containing oligosaccharides besides N-e. (2) Although experimental coordinates are not available for M-d, the coordinates for an oligosaccharide can be computed from "unit contribution" values with reasonable accuracy [22]. The computed coordinate for the structure desialyl M-d is (14.8, 6.9), which is close to the observed value for desialyl M-d (15.5, 6.9).

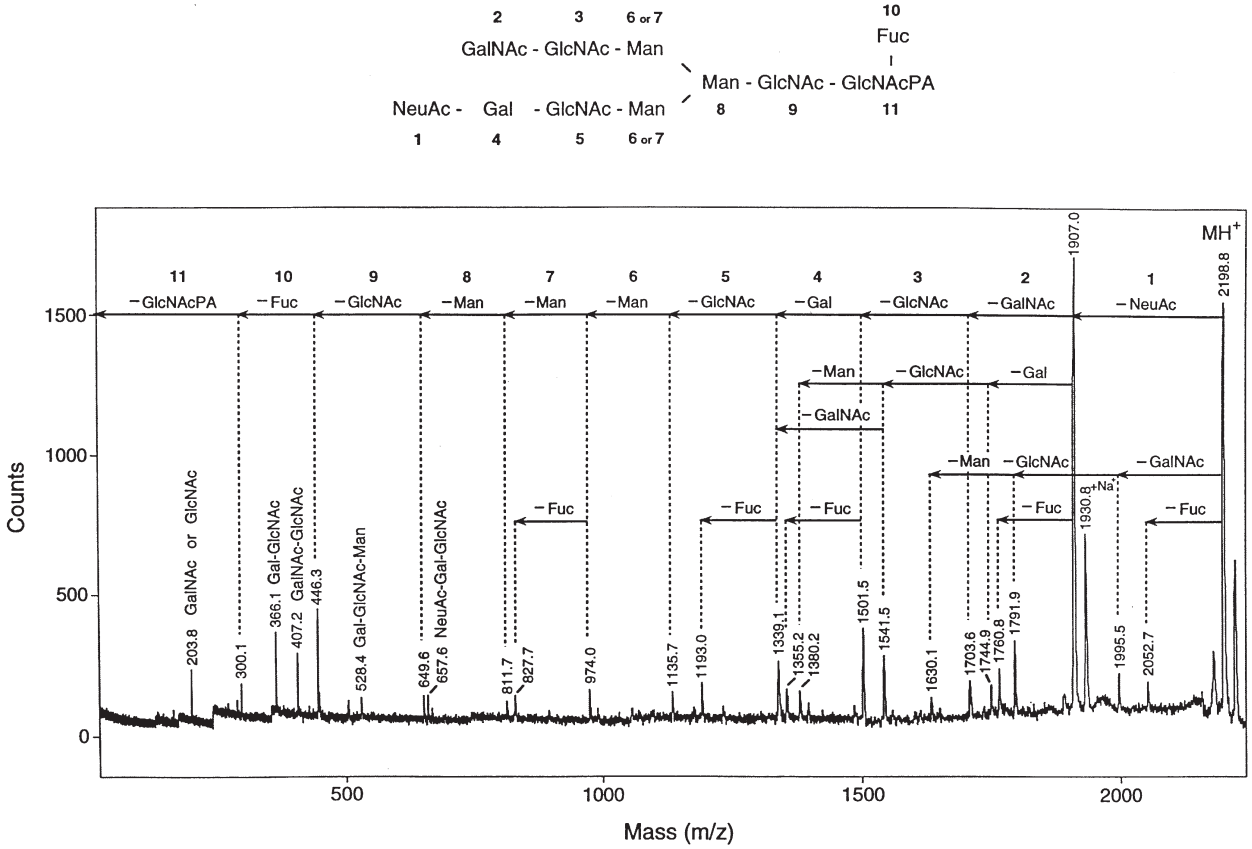
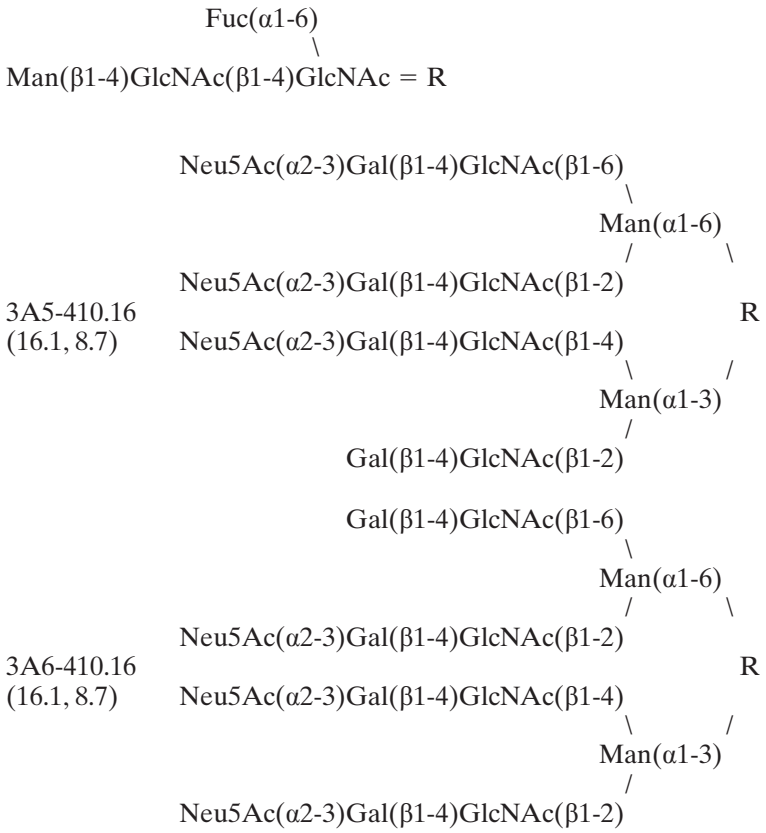


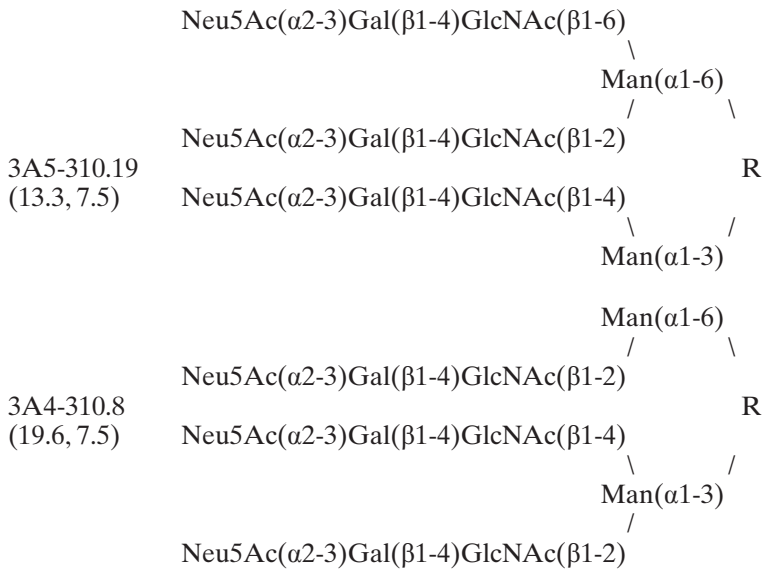
Figure 3. PSD-MALDI mass spectrum of PA-oligosaccharide M-d. MH^+ ion was selected as the parent ion. The spectrum was measured by reflector mode.

Identification of T-c1 and T-c2

Fraction T-c (16.1, 8.7, on the 2-D map) was confirmed to originate from a typical neutral *N*-glycan 410.16 (tetra-antennary with fucose residue at the reducing end *N*-acetylglucosamine). Based on the coordinate value of T-c, possible structures of tri-sialylated 410.16 were limited to the following [17]:



The above two structures can not be separated on the 2-D map directly. Following β-galactosidase and β-*N*-acetylhexosaminidase digestion, however, T-c fraction was converted into two fractions, as below, which can be unambiguously identified on ODS column.



The original T-c fraction was thus shown to be a 1:2 mixture of 3A5-410.16 and 3A6-410.16.

All separated and characterized *N*-glycans from mouse FcγR are listed in Table 1. These *N*-glycans expressed in BHK cells include significant amounts of paucimannoside structures. N-a (13.8%), N-b (6.3%), N-cl (11.8%), and M-b (12.7%) are not intermediates in the oligosaccharide synthetic pathway established for animal cells [23, 24]. Since degradation of sugar chains during analytical procedures is unlikely, these paucimannoside structures may be formed by trimming of larger oligosaccharides through digestion by unidentified exoglycosidases after completion of mature glycoprotein synthesis.

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