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SHORT COMMUNICATION

Evidence for a site-specific fucosylation of *N*-linked oligosaccharide of immunoglobulin A1 from normal human serum

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Glycopeptides containing the *N*-linked oligosaccharide from human serum IgA1 were analyzed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS). Two glycopeptides, GP1 and GP2, prepared from the endoproteinase Asp-N digest of the IgA1 heavy chain, were derived from the CH2 domain (*N*-glycan site at Asn^{263}) and the tailpiece portion (*N*-glycan site at Asn^{459}), respectively. The structure of the attached sugar chain was deduced from the mass number of the glycopeptide and confirmed by a two-dimensional mapping technique for a pyridylaminated oligosaccharide. GP1 was composed of two major components having a fully galactosylated bianntena sugar chain with or without a bisecting *N*-acetylglucosamine (GlcNAc) residue. On the other hand, the GP2 fraction corresponded to the glycopeptides having a fully galactosylated and fucosylated bianntena sugar chain partly bearing a bisecting GlcNAc residue. Thus, the site-specific fucosylation of the *N*-linked oligosaccharide on the tailpiece of the α1 chain became evident for normal human serum IgA1.

Keywords: **human serum IgA1, site-specific fucosylation, *N*-linked oligosaccharide, MALDI-TOFMS, two-dimensional mapping**

Abbreviations: **The abbreviations and trivial names used are IgA1, immunoglobulin A1; MALDI-TOFMS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; HPLC, high-performance liquid chromatography; GlcNAc, *N*-acetylglucosamine; Hex, hexose; HexNAc, *N*-acetylhexosamine; deoxyHex, deoxyhexose; Fuc, fucose; Gal, galactose; Man, mannose; TFA, trifluoroacetic acid; PA, pyridyl-2-amino; 2-D mapping, two-dimensional mapping**

Introduction

Human serum IgA1 is one of the most exceptional proteins among the human serum glycoproteins because it has *O*-linked oligosaccharides in its hinge portion in addition to two *N*-linked carbohydrate chains in its heavy chain [1–3]. In our previous study, a hinge glycopeptide was purified from a tryptic digest of IgA1 by HPLC, and the glycoforms of the *O*-linked oligosaccharides of the IgA1 were analyzed by gas-phase hydrazinolysis and MALDI-TOFMS [4–7].

With respect to the *N*-linked oligosaccharide, structural analysis was first carried out by Baenziger and Kornfeld [1], and, recently, an extensive study on the *N*-glycan sugar chain of IgA1 and IgA2 from many myeloma patients was performed by Endo et al. [8]. Research by Field et al. [9] determined the actual structures of the *N*-linked oligosaccharides in normal human serum IgA1. The majority of *N*-linked oligosaccharides was found to be the disialylated biantennary-complex-type [9]. Wormald et al. [10] reported a site-specific glycosylation profile of IgG and discussed

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the relationship to its normal physiologic role and the role in rheumatoid arthritis. Differing from IgA, almost all sugar chains on both sites of IgG were fucosylated. Species specific fucosylation of the *N*-glycan sugar chain of the plasminogen was also reported by Marti et al. [11].

In this report, the site-specific structural difference in the *N*-linked oligosaccharide of normal human serum IgA1 was conveniently examined without release of the oligosaccharide from the protein portion. The obtained site-specific fucosylation in normal human serum IgA1 was different from the recent report on the recombinant IgA1 by Mattu et al. [12].

Experimental procedure

Materials

The following materials were purchased from the indicated sources: sialidase from *Arthrobacter ureafaciens* from Boehringer Mannheim (Germany); β-galactosidase from *Jack bean* from Seikagaku Co. (Tokyo, Japan); α-L-fucosidase from *bovine epididymis* from Sigma Chem. Co. (St. Louis, Missouri, USA); Endoproteinase Asp-N from *Pseudomonas fragi* and PA-oligosaccharide standards (001, 009) from Takara Shuzo Co., Ltd. (Shiga, Japan).

Preparation of the glycopeptides, GP1 and GP2, from the S-pyridylethylated α1 chain

Preparation of the S-pyridylethylated α1 chain from human serum IgA1 was carried out as reported previously [6]. About 1 mg of the S-pyridylethylated α1 chain was incubated with 2 μg of endoproteinase Asp-N in 50 mM sodium phosphate buffer, pH 8.0 for 3 days at room temperature. The glycopeptides were fractionated by HPLC on a Cosmosil 5C18-300 column (4.6 × 150 mm). Elution was carried out with a linear gradient for 60 min from 0% to 90% acetonitrile in 0.1% TFA. Detection was performed by UV absorption monitoring at 220 nm. The glycopeptides, GP1 which eluted at a peak position around 30 min and GP2 which eluted at a peak position around 24 min, were collected and concentrated. The purified glycopeptides were digested overnight at room temperature with 50 mU of sialidase in 0.2 M sodium acetate buffer, pH 5.0. These asialo glycopeptides were then treated with 4.0 mU of β-galactosidase or 1.6mU of α-L-fucosidase under the optimum conditions, overnight at room temperature.

MALDI-TOFMS analysis and amino acid sequence analysis of the glycopeptide

The glycopeptide was analyzed by MALDI-TOFMS in the positive ion mode. The mass spectrometer used in this work was a Finnigan Lasermat (Finnigan MAT, Ltd., Hemel Hempstead, UK). The matrix used was α-cyano-4-hydroxy cinnamic acid (10 mg/ml) in a 70% acetonitrile solution. The error involved in the mass determination was less than 0.3%. Amino acid sequence analyses were performed using an Applied Biosystems Procise 492 Protein Sequencer (Perkin-Elmer, California, USA).

Two-dimensional elution mapping of pyridylaminated *N*-linked oligosaccharide from IgA1

Preparation of the oligosaccharide from IgA1, the α1 chain, GP1, and GP2 was carried out by gas-phase hydrazinolysis using Hydraclub S-204 as previously reported [4, 13]. Released oligosaccharide was reductively aminated with 2-aminopyridine according to the methods reported by Hase [14]. 2-D mapping was carried out under the standard conditions as reported before [15].

Results and discussion

The peptide fractions separated by HPLC were analyzed by MALDI-TOFMS and *N*-terminal amino acid sequence analysis. From these results and the previously reported amino acid sequence for IgA1 Bur [16], two glycopeptides, GP1 and GP2, were identified (data not shown); that is, GP1 and GP2 were derived from the CH2 domain including the Asn^{263} residue and the tailpiece portion containing the Asn^{459} residue, respectively.

The m/z values of peaks A and B from asialo GP1 were identical to the calculated average mass of (Hex5•HexNAc4)-peptide and (Hex5•HexNAc5)-peptide, respectively (Figure 1A; Table 1). From these results, the m/z value for the glycosidase-treated glycopeptide in Table 1 and the previously reported carbohydrate chain structures for IgA1, the structure of the carbohydrate moiety of asialo GP1, was presumed to be composed of two major components having a fully galactosylated bianntena sugar chain with or without a bisecting GlcNAc residue.

On the other hand, sialidase-treated GP2 was further separated into asialo GP2-1 and asialo GP2-2 by HPLC (data not shown). The sequence analysis indicated that the peptide portion of asialo GP2-2 was shorter than the (DRLAGKPTHVXVSVVMA) of asialo GP2-1 by one alanine residue. The calculated average mass of the two major peaks, a′ and b′, in Figure 1C corresponded to the (Hex5•HexNAc4•deoxyHex1)-peptide and the (Hex5•HexNAc5•deoxyHex1)-peptide, respectively. As summarized in Table 1, two galactose residues and a fucose residue were released from GP2-2 by the treatment with β-galactosidase or α-L-fucosidase. Based on these results, the structure of the carbohydrate moiety of asialo GP2 was estimated to be composed of two major components having a fully galactosylated fucosylated bianntena sugar chain and the same sugar chain bearing a bisecting GlcNAc. Thus, most of the sugar chains of GP1 and GP2 were fully galactosylated, and a part of them contained a bisecting GlcNAc

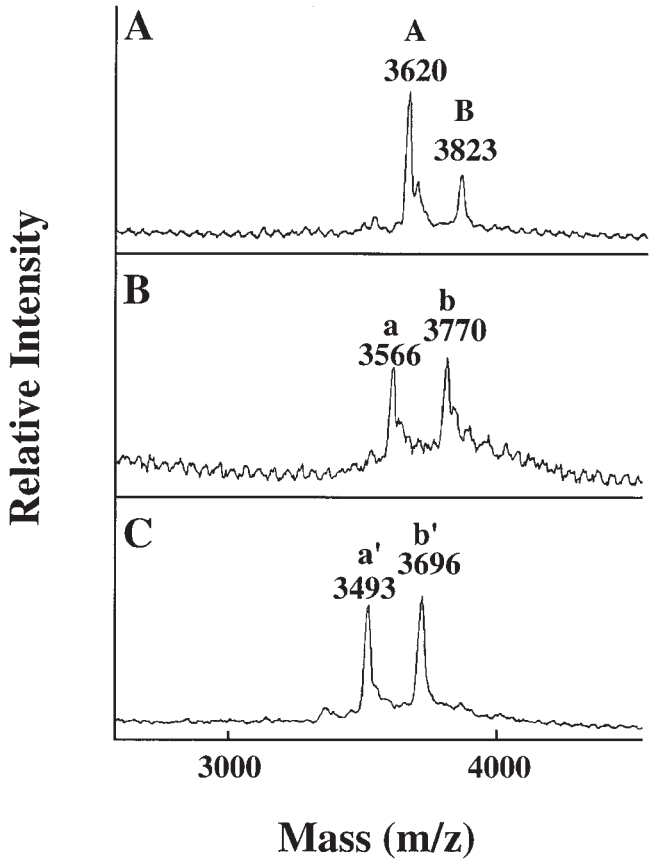

Figure 1. MALDI-TOFMS analysis of the asialo glycopeptides A, Asialo GP1; B, Asialo GP2-1; C, Asialo GP2-2. MALDI-TOFMS analysis was carried out under the conditions given under "Experimental procedure." The m/z value for the major ion is indicated in the figure. The structure of the peptide moiety of GP1 and GP2-2 was identified as DLLLGSEAXLTCTLTGLR and DRLAGKPTHVXVSVVMA (X was probably an asparagine residue which was attached to a sugar chain) by amino acid sequence analysis, respectively.

residue; however, the fucose residue was present only on GP2 and not on GP1.

To confirm these deduced structures for the carbohydrate chain, oligosaccharides released from the samples were analyzed by the 2-D mapping technique. As summarized in Figure 2, six oligosaccharides were prepared from IgA1. Among them, A and E were relatively abundant in GP1, and D and F were abundant in GP2. These combinations of the carbohydrate structures, A, E and D, F as shown in Table 2 coincided with the carbohydrate structures for GP1 and GP2 deduced from their mass numbers. Thus, the site-specific fucosylation of the *N*-linked oligosaccharide on the tailpiece of the α1 chain became evident for normal human serum IgA1.

There are many reports on aberrant glycosylation of IgA1 in IgA nephropathy patients with respect to *O*- and *N*-linked oligosaccharides [17-22]. Application of this method to IgA1 from an IgA nephropathy patient indicated that individual samples showed the same site-specific fucosylation and no differences in its mass number between the major asialo glycopeptides derived from IgA1 of the controls and the patients (data not shown). Therefore, the obtained site-specific fucosylation would be general and reproducible for human serum IgA1. There were two controversial reports in which the *N*-linked oligosaccharide on the tailpiece was related with the dimer assembly of IgA1 molecule [23, 24]. Recently, Mattu et al. [12] reported that the site-specific structural difference of the *N*-glycan sugar chain of the recombinant IgA1 showed the presence of a tailpiece bearing a triantennary structure. Thus, this result was different from ours. The reason why the site- specific fucosylation or site-specific trianntenary construction occurred is unclear, but such a structural difference may play an important role in the function of human serum IgA1 which is produced only in primates, including humans [25].

Table 1. Mass numbers of asialo glycopeptides treated with β-galactosidase or α-L-fucosidase

		Mass Number	
Glycopeptide	*Treatment*	*Calculated [M + H]⁺*	*Observed (m/z)*
Asialo GP1-A		3619.8	3620
	β-Galactosidase	3295.5	3297
Asialo GP1-B		3823.0	3823
	β-Galactosidase	3498.7	3501
Asialo GP2-2-a′		3493.6	3493
	β-Galactosidase	3169.4	3175
	α-L-Fucosidase	3347.5	3353
Asialo GP2-2-b′		3696.8	3696
	β-Galactosidase	3372.6	3377
	α-L-Fucosidase	3550.7	3556

GP1-A, -B, and GP2-2-a′, -b′ are indicated in Figure 1.

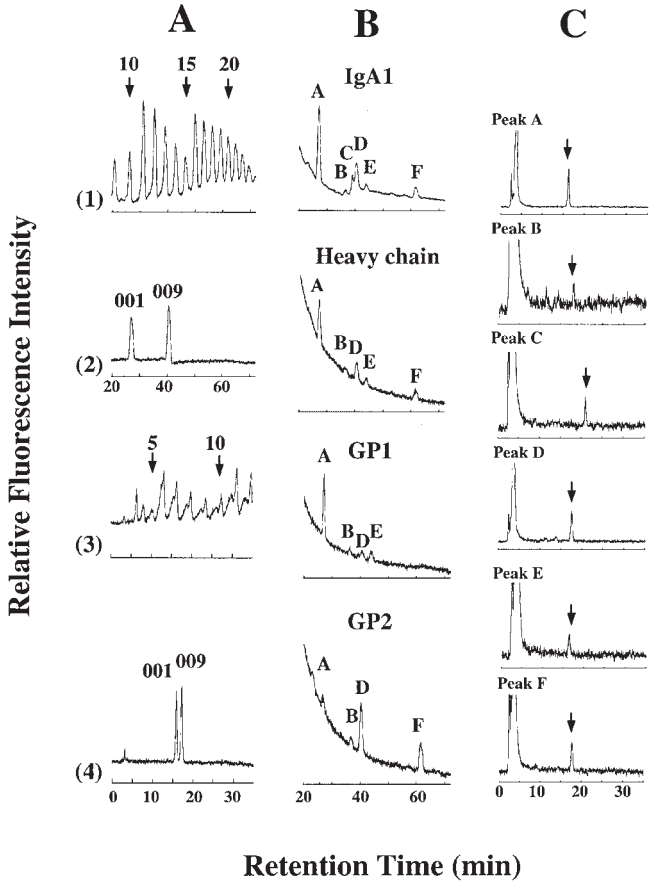

Figure 2. HPLC profiles of PA-oligosaccharide prepared from IgA1 for two-dimensional mapping analysis Figure A: ODS (1) and (2) and Amide-80 (3) and (4) column chromatography of PA-malto-oligosaccharides (1) and (3) and PA-standard *N*-glycan oligosaccharides (2) and (4). Number of glucose units in maltooligosaccharide standards, 001 and 009, are indicated in (1) and (3). Structures of the standard oligosaccharides 001 and 009 in (2) and (4) are indicated in Table 2. Figure B: Elution profiles on ODS column of PA-oligosaccharides obtained from IgA1, heavy chain, GP1 and GP2. Peaks A-F on a first dimensional HPLC were collected and applied to a second dimensional HPLC as shown in the figure C. Figure C: Peaks A-F in Figure 2B were applied to HPLC using Amide-80 column. Arrow indicates eluted position of each PA-oligosaccharide. ab

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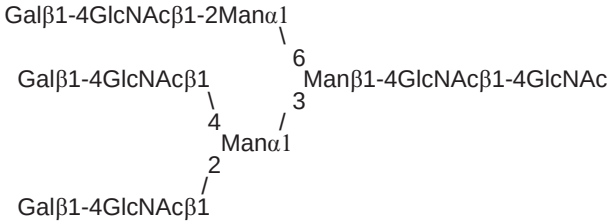
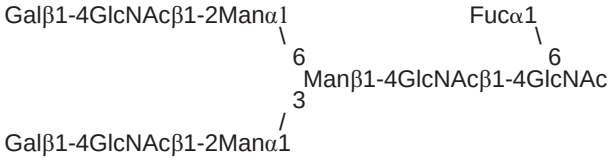
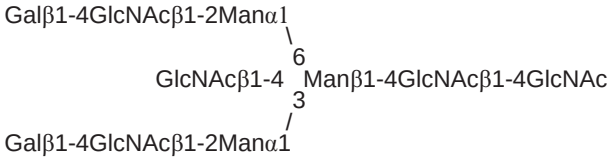
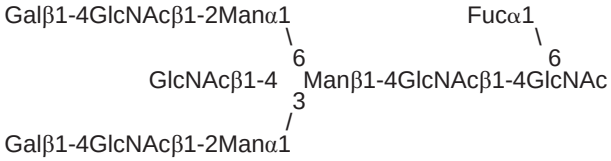
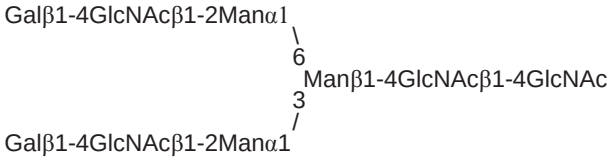
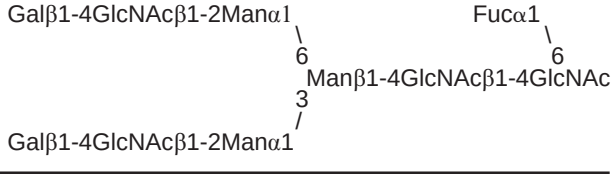
Table 2. Determination of structures of *N*-glycan sugar chain on IgA1 by two-dimensional mapping technique

	Glucose unit		
Oligosaccharide	*ODS*	*Amide*	*Reported Structure*[a]
A	10.1[b] (10.2)[c]	6.9 (7.0)	Galβ1-4glcNAcβ1-2Manα1 \ 6 Manβ1-4GlcNAcβ1-4GlcNAc 3 / Galβ1-4GlcNAcβ102Manα1

Table 2. (continued)

Oligosaccharide	*Glucose unit* ODS	*Glucose unit* Amide	*Reported Structure*[a]
B	12.5	7.5	**Unknown**
C	13.0 (12.7)	8.3 (8.3)	
D	13.4 (14.1)	7.4 (7.4)	
E	14.3 (14.5)	7.1 (7.2)	
F	19.9 (20.2)	7.5 (7.5)	
Standard			*Structure*
001	10.3	6.9	
009	13.5	7.3	

[a]Reported in reference [15].
[b]Observed values.
[c]Value reported in reference [15] are in parentheses.

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