

A comparative study of the *N*-linked oligosaccharide structures of human IgG subclass proteins

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Quantitative oligosaccharide profiles were determined for each of 18 human IgG paraproteins representing the four subclasses. Each paraprotein exhibits a unique profile that may be substantially different from that observed for polyclonal IgG. The IgG2 and some IgG3 proteins analysed exhibit a predominance of oligosaccharide moieties having galactose on the Man(α 1→3) arm rather than the Man(α 1→6) arm; it was previously held that galactosylation of the Man(α 1→6) arm is preferred, as observed for IgG1, IgG4 and polyclonal IgG. An IgG4 protein is reported that has galactosylated Man(α 1→3) and Man(α 1→6) arms on both Fc-localized carbohydrate moieties; previous findings suggested that such fully glycosylated structures could not be accommodated within the internal space of the C₂ domains. Unusual monoantennary oligosaccharides present in IgG2 and IgG3 proteins were isolated and their structures determined.

INTRODUCTION

Each human immunoglobulin isotype has a characteristic glycosylation profile that may include multiple complex oligosaccharide moieties attached to asparagine residues or simple disaccharides attached through serine or threonine side chains (Pumphrey, 1986). Particular interest is attached to the complex oligosaccharide attached to asparagine-297 of the human IgG gamma heavy chain, which is enclosed between the C₂ domains and is an integral part of the tertiary/quaternary structure of the Fc region (Deisenhofer, 1981; Sutton & Phillips, 1983). Aglycosylated IgG, obtained by culturing IgG-producing cell lines in the presence of tunicamycin, has been shown to lack several important biological functions, e.g. binding to monocyte Fc_γRI receptor (Leatherbarrow *et al.*, 1985; Walker *et al.*, 1989), to display decreased complement activation (Nose & Wigzell, 1983; Leatherbarrow & Dwek, 1983) and to display increased susceptibility to proteolysis (Leatherbarrow & Dwek, 1983). However, glycopeptides isolated after proteolytic digestion of IgG have not been demonstrated to have biological activity, e.g. binding to Fc receptors, C1 etc. An indirect role is suggested by the intimate involvement of the oligosaccharide with the protein structure; the unpaired C₂ domains have hydrophobic amino acid side chains (potentially) exposed to the aqueous medium; however, interaction with the oligosaccharide moiety might prevent such contact. An additional contribution to quaternary structure results from trans interaction(s) between the carbohydrate moieties on each heavy chain. The symmetry of the Fc region requires the two oligosaccharide chains to be accommodated within the restricted space available between the C₂ domains, and it has been suggested that, for rabbit IgG, this is not possible if each chain is galactosylated (Sutton & Phillips, 1983). A variety of incompletely glycosylated chains can be accommodated in various combinations, resulting in considerable heterogeneity, as observed for polyclonal IgG and monoclonal IgG. The intimate association of the carbohydrate with the

protein structure suggests that such structural heterogeneity (glycoforms) may result in functional heterogeneity. The development of analytical techniques for determining oligosaccharide profiles of IgG molecules has allowed definition of species-distinct glycosylation patterns and revealed heterogeneity of glycosylation within monoclonal proteins (Mizuochi *et al.*, 1982; Rademacher & Dwek, 1983). Differences in oligosaccharide profiles have been observed between polyclonal IgG isolated from the serum of normal individuals and that from patients with inflammatory diseases (Parekh *et al.*, 1985; Axford *et al.*, 1987; Tomana *et al.*, 1988).

We have applied a recently developed 'mapping' technique (Takahashi *et al.*, 1987; Tomiya *et al.*, 1988) to the analysis of the oligosaccharide structures of a panel of IgG paraproteins representing each subclass. Although each protein shows heterogeneity, its oligosaccharide profile was unique, suggesting that each clone may be characterized by its glycosyltransferase activity. Proteins of the same subclass but differing radically in their glycosylation profiles have been identified (Jefferis *et al.*, 1988).

MATERIALS AND METHODS

Enzymes and standard oligosaccharides

N-Oligosaccharide glycopeptidase from almond (obtainable as Glycopeptidase A), β -galactosidase from *Charonia lampas*, β -*N*-acetylhexosaminidase from jack bean and endo- β -*N*-acetylglucosaminidase D from *Diplococcus pneumoniae* were purchased from Seikagaku Kogyo Co. α -L-Fucosidase from bovine kidney was purchased from Boehringer Mannheim Biochemicals. Pepsin was purchased from Sigma Chemical Co.

Standard biantennary and monoantennary oligosaccharides were prepared from normal IgG by the method of Takahashi *et al.* (1987) and Tomiya *et al.* (1988). The pyridylamino derivatives of the oligosaccharides were prepared and purified as described

Abbreviations used: Fuc or F, L-fucose; Gal or G, D-galactose; GlcNAc or GN, *N*-acetyl-D-glucosamine; Man or M, D-mannose; PA, pyridylamino.

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previously (Hase *et al.*, 1984). Gal β 1 \rightarrow 4GlcNAc β 1 \rightarrow 2Man α -1 \rightarrow 6(Man α 1 \rightarrow 3)Man β 1 \rightarrow 4GlcNAc β 1 \rightarrow 4(Fuca1 \rightarrow 6)GlcNAc-PA (no. 110.3) and Man α 1 \rightarrow 6(Gal β 1 \rightarrow 4GlcNAc β 1 \rightarrow 2Man α -1 \rightarrow 3)Man β 1 \rightarrow 4-GlcNAc β 1 \rightarrow 4(Fuca1 \rightarrow 6)GlcNAc-PA (no. 110.4) were prepared from polyclonal IgG oligosaccharides F and G respectively by digestion with β -N-acetylglucosaminidase (Takahashi *et al.*, 1987). GlcNAc β 1 \rightarrow 2Man α 1 \rightarrow 6(Man α 1 \rightarrow 3)Man β 1 \rightarrow 4GlcNAc β 1 \rightarrow 4(Fuca1 \rightarrow 6)GlcNAc-PA (no. 110.1) and Man α 1 \rightarrow 6(GlcNAc β 1 \rightarrow 2Man α 1 \rightarrow 3)Man β 1 \rightarrow 4GlcNAc β -1 \rightarrow 4(Fuca1 \rightarrow 6)GlcNAc-PA (no. 110.2) were prepared from pyridylamino-oligosaccharides nos. 110.3 and 110.4 respectively by digestion with β -galactosidase. Gal β 1 \rightarrow 4GlcNAc β 1 \rightarrow 2Man α -1 \rightarrow 6(Man α 1 \rightarrow 3)Man β 1 \rightarrow 4GlcNAc β 1 \rightarrow 4GlcNAc-PA (no. 100.3) and Man α 1 \rightarrow 6(Gal β 1 \rightarrow 4GlcNAc β 1 \rightarrow 2Man α 1 \rightarrow 3)-Man β 1 \rightarrow 4GlcNAc β 1 \rightarrow 4GlcNAc-PA (no. 100.4) were prepared from pyridylamino-oligosaccharides nos. 110.3 and 110.4 respectively by digestion with α -L-fucosidase.

Other chemicals

The following materials were purchased from the sources indicated: human polyclonal IgG proteins from United States Biochemical Co., Sephadex G-15 from Pharmacia, Bio-Gel P-4 (200–400 mesh) from Bio-Rad Laboratories, sodium cyanoborohydride from Aldrich Chemical Co. and 2-aminopyridine from Wako Pure Chemical Industries.

Preparation of human IgG subclass proteins

IgG paraproteins were isolated from the sera of patients with multiple myeloma, with an accompanying hypogammaglobulinaemia resulting in polyclonal IgG being diminished to background levels. Sera were dialysed into 0.01 M-sodium phosphate buffer, pH 7.0, and eluted as the breakthrough fraction from DEAE-cellulose equilibrated with the same buffer. Some IgG4 proteins bound to the exchanger under these conditions and were eluted with 0.03 M-sodium phosphate buffer, pH 7.0. To generate Fab and Fc fragments of IgG4 proteins the purified intact immunoglobulins were dialysed against 0.1 M-sodium phosphate buffer, pH 7.0, containing 1 mM-dithiothreitol and 2 mM-EDTA. After digestion with activated (with 1 mM-dithiothreitol for 30 min at 37 °C) papain (2%, w/w) at 37 °C for 2 h, digestion

was terminated by the addition of iodoacetamide (10 mM). The digest was dialysed against 0.01 M-sodium phosphate buffer, pH 7.3, and passed over a DE52 DEAE-cellulose (Whatman) column equilibrated with the same buffer. The Fab fragment does not bind under these conditions and was recovered in the

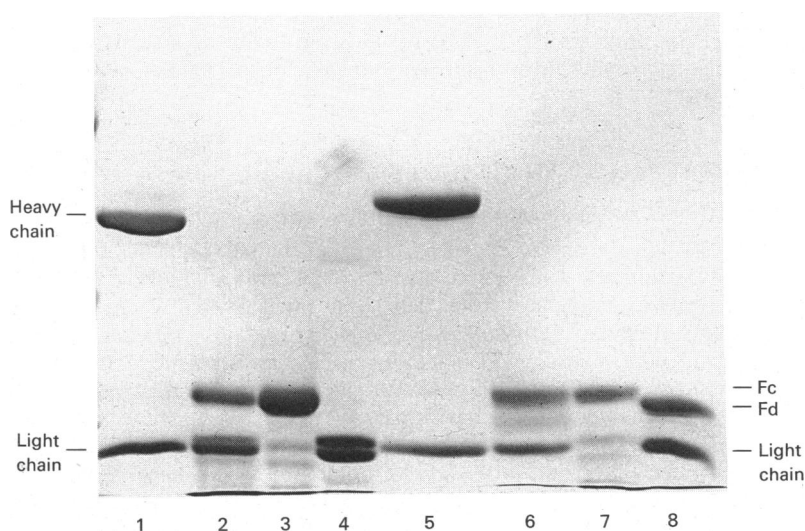
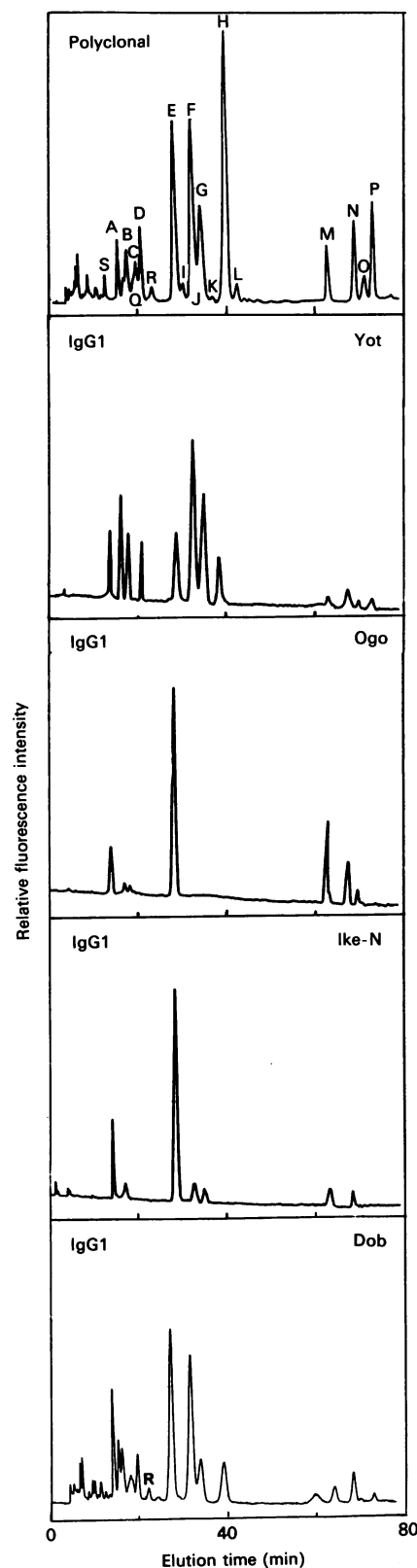


Fig. 1. SDS/PAGE analysis (10% gel) under reducing conditions of intact IgG4 proteins Rea and AS (tracks 1 and 5), the unseparated papain digests (tracks 2 and 6) and their respective purified Fc and Fab fragments (tracks 3 and 7 and tracks 4 and 8)



'wash-through' fraction; the Fc was eluted with 0.01 M-sodium phosphate buffer, pH 7.3, containing 0.15 M-NaCl.

The purity of protein preparations was checked by SDS/PAGE analysis on overloaded (approx. 25 µg) gels. Intact proteins gave a single band under non-reducing conditions and two bands corresponding to heavy chains and light chains on reducing gels. Representative results for the IgG4 proteins Rea and AS and their Fab and Fc fragments are shown in Fig. 1.

Preparation of oligosaccharides from IgG

A 5–10 mg portion of each of the IgG samples was used for the isolation of oligosaccharides. IgG was quantitatively desialylated by mild acid hydrolysis at pH 2 (0.01 M-HCl) for 1 h at 90 °C. Desialylated and pepsin-digested IgG glycopeptides were treated with *N*-oligosaccharide glycopeptidase as described previously (Takahashi *et al.*, 1987). The oligosaccharide fraction was collected by gel filtration on a Bio-Gel P-4 column and further purified by passing it through columns of ion-exchange resins Dowex 50W X8 (H⁺ form) and Amberlite IRA-400 (CO₃²⁻ form) (Nishibe & Takahashi, 1981).

Isolation of pyridylamino-oligosaccharides by h.p.l.c.

Oligosaccharide fractions obtained as above were reductively aminated with 2-aminopyridine by the use of sodium cyanoborohydride (Hase *et al.*, 1984). The pyridylamino derivatives of oligosaccharides purified by gel filtration on a Sephadex G-15 column were fractionated and identified by h.p.l.c. with the use of a two-dimensional mapping technique as described previously (Tomiya *et al.*, 1988). Two kinds of column were used, namely a Shimpack CLC-ODS column (0.6 cm × 15 cm) and a TSKgel Amide-80 column (0.46 cm × 25 cm).

Analytical procedures

Sample proteins were assayed by the orcinol/H₂SO₄ reaction to provide a minimum estimate of neutral sugar content (François *et al.*, 1962); mannose, galactose and, to a lesser extent, fucose contribute to the colour yield, whereas the neutral sugars glucosamine and galactosamine do not. Sialic acids were determined by the method of Hara *et al.* (1987). The relative quantity (%) of each component oligosaccharide (A–S) as a proportion of the total carbohydrate of human polyclonal IgG

Table 1. Sialic acid and neutral sugar contents of IgG proteins

	Neutral sugar/ protein ratio (mol/mol)	Sialic acid/ protein ratio (mol/mol)	Sialic acid/ neutral sugar ratio (mol/mol)
Polyclonal	7.91	0.59	0.075
IgG1 Ike-N	5.95	0.16	0.026
IgG1 Ogo	7.28	0.23	0.032
IgG2 Ku3	8.48	0.11	0.013
IgG2 INK	6.60	0.31	0.047
IgG3 Jir	9.89	0.35	0.035

and subclass IgG (Table 2) was determined by integrating the area under each peak and summing these values to determine that for the total carbohydrate content.

RESULTS

Purity of IgG paraproteins, Fab and Fc fragments

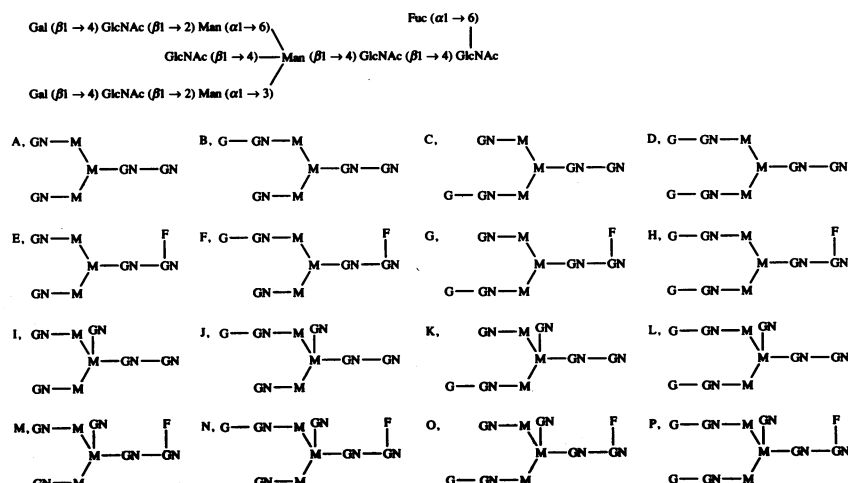
Results obtained on overloaded SDS/10 %-PAGE gels run under reducing conditions are shown in Fig. 1. The intact proteins yield heavy-chain and light-chain bands only. The Fc fragments have minor components that are due to papain cleavage at secondary sites within the Fc region. The Fab fragments are essentially pure, yielding light chain and the Fd fragment of the heavy chain.

Oligosaccharide profiles of monoclonal IgG of each subclass

The *N*-acetylneuraminic (sialic) acid and (minimum) neutral sugar contents of sample proteins are given in Table 1. The minimum neutral sugar content observed is six residues, consistent with molecules having oligosaccharide moieties on each heavy chain devoid of galactose and fucose, i.e. the orcinol/H₂SO₄ reaction detects the three mannose residues present on each chain only. Sialic acid constitutes a minor proportion of the total sugar content of polyclonal IgG and a lesser proportion for the monoclonal proteins analysed. Detailed oligosaccharide profiles were therefore determined on proteins treated under

Fig. 2. H.p.l.c. profiles of pyridylamino derivatives of oligosaccharide derived from normal IgG (polyclonal) and IgG subclass 1 paraproteins (Yot, Ogo, Ike-N and Dob)

Oligosaccharide fractions obtained by glycopeptidase digestion of the peptic peptides of IgG were aminated and subjected to h.p.l.c. analyses as described in the text. Symbols: G, galactose; M, mannose; F, fucose and GN, *N*-acetylglucosamine. Peak P, for example, represents:



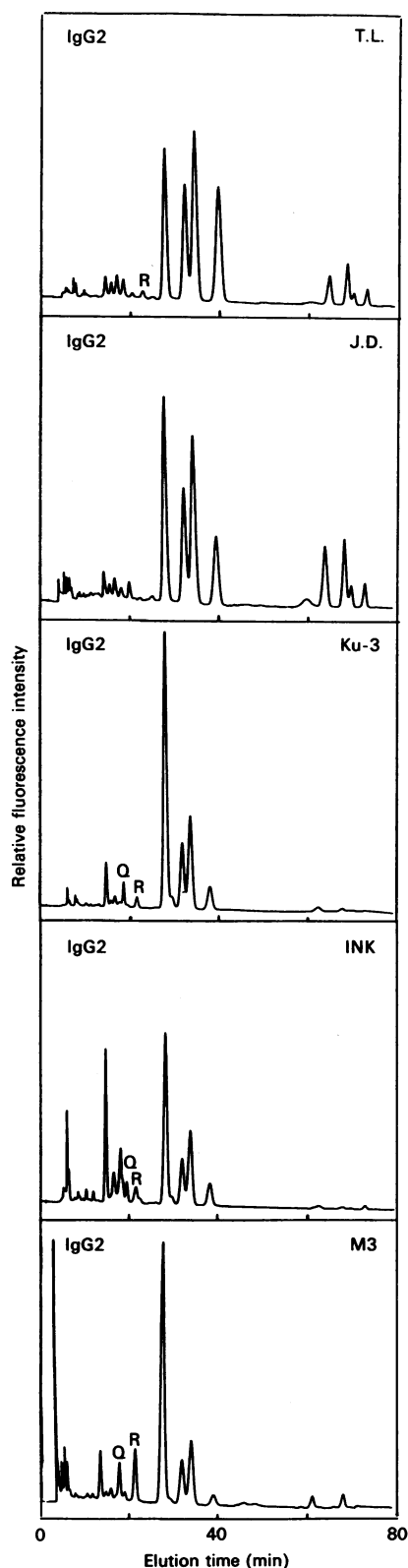


Fig. 3. Comparison of h.p.l.c. profiles of pyridylamino derivatives of oligosaccharides derived from human IgG2 paraproteins T.L., J.D., Ku-3, INK and M3

mild acid conditions to remove sialic acid and simplify the data for interpretation.

Oligosaccharides were released by sequential digestion of IgG subclass proteins (5–10 mg) with pepsin and *N*-oligosaccharide

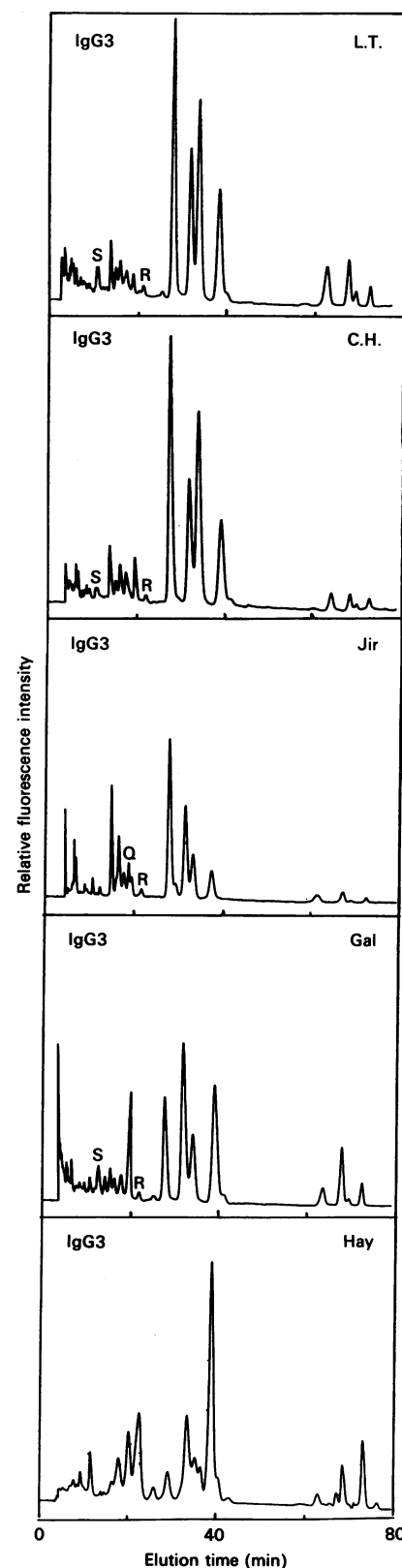


Fig. 4. Comparison of h.p.l.c. profiles of pyridylamino derivatives of oligosaccharides derived from human IgG3 paraproteins L.T., C.H., Jir, Gal and Hay

glycopeptidase (almond); total carbohydrate analysis showed that less than 10% remained as undigested material (results not shown). The oligosaccharide profiles obtained on octadecyl-

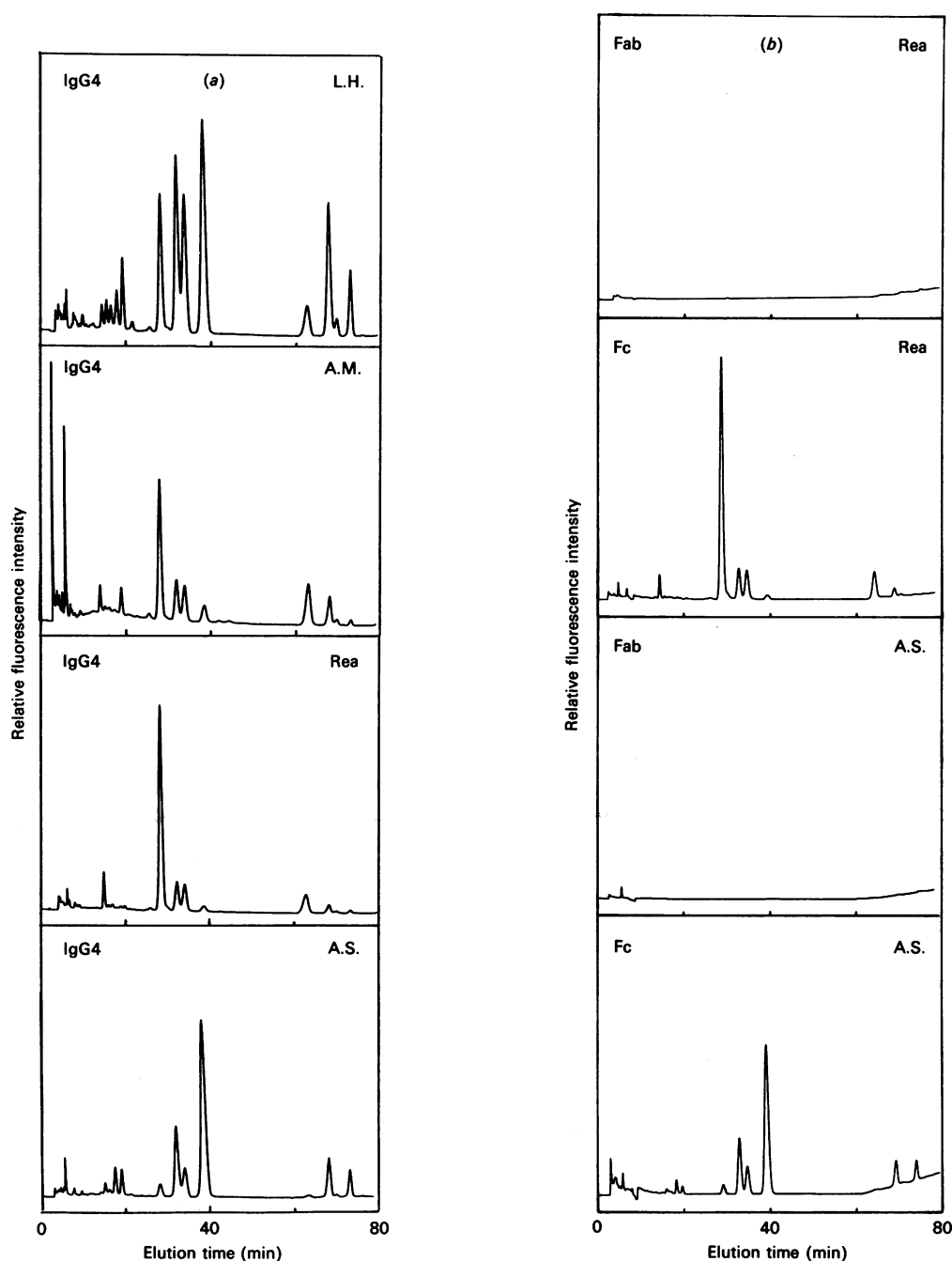


Fig. 5. Comparison of h.p.l.c. profiles of pyridylamino derivatives of oligosaccharides derived from (a) human IgG4 paraproteins L.H., A.M., Rea, and A.S. and (b) Fab and Fc fragments derived from proteins Rea and A.S.

silica (ODS) columns for IgG proteins from each of the four subclasses are shown in Figs. 2–5. Also included in the top of Fig. 2 is the oligosaccharide profile obtained from polyclonal IgG. High-resolution ^1H -n.m.r. spectroscopy was used together with chemical methods to determine the detailed structures of the separated oligosaccharides A–P in polyclonal IgG (Takahashi *et al.*, 1987). The elution positions of polyclonal oligosaccharides of group I (A–D), group II (E–H), group III (I–L) and group IV (M–P) are indicated. These groups refer to oligosaccharides that are biantennary without fucose, biantennary with fucose, bi-antennary with bisecting *N*-acetylglucosamine and without fucose and biantennary with bisecting *N*-acetylglucosamine and fucose respectively. Assignments of structures to each of these oligosaccharides are summarized in the legend to Fig. 2. These

authentic oligosaccharides were prepared from polyclonal IgG proteins.

It is evident that group II oligosaccharides predominate, with group IV oligosaccharides contributing 2–40% of the total (Table 2). It is also of interest to note the wide variation in the proportions of the individual group II oligosaccharides within different proteins. Thus the IgG4 proteins Rea and A.S. have 62% of component E [lack of galactose on both the $\text{Man}(\alpha 1 \rightarrow 3)$ and $\text{Man}(\alpha 1 \rightarrow 6)$ arms] and 52% of component H [galactose present on both the $\text{Man}(\alpha 1 \rightarrow 3)$ and $\text{Man}(\alpha 1 \rightarrow 6)$ arms] respectively. In addition, protein A.S. contains components D (3.7%) and P (6.4%) (Fig. 2), resulting in 62% of oligosaccharide moieties having galactose on each arm while 38% of oligosaccharide moieties have galactose on one arm only or galactose

Table 2. Relative quantity of each component oligosaccharide (A-S) as a percentage of the total carbohydrate of human polyclonal and subclass IgG

The percentage of each component oligosaccharide (A-S) was calculated by its peak area on the h.p.l.c. profile since the relative fluorescence is the same on a molar basis for each component (Hase *et al.*, 1984).

Component oligo-saccharide	Relative quantity (%)															
	IgG1				IgG2				IgG3				IgG4			
	Polyclonal	Yot	Ogo	Ike-N	Dob	T.L.	J.D.	KU-3	INK	M3	L.T.	C.H.	Jir	Gal	Hay	A.S.
A	2.6	6.2	10.0	11.9	3.4	-	-	1.7	15.5	-	-	-	11.0	-	-	-
B	2.7	10.7	-	5.9	3.3	-	-	0.5	5.4	-	-	-	8.4	-	4.9	-
C	2.1	6.9	-	-	-	-	-	-	7.1	-	-	-	3.7	-	-	-
D	4.7	4.7	-	-	5.4	0.4	1.2	-	2.4	-	1.7	3.8	2.4	10.1	16.2	3.7
E	13.9	10.9	52.7	59.6	28.8	20.5	24.3	50.8	30.4	56.3	26.1	30.8	26.9	13.6	4.6	61.7
F	15.1	25.9	-	7.9	29.6	17.6	17.5	15.2	10.4	10.7	16.7	16.4	18.1	25.5	14.1	17.4
G	9.7	18.5	-	5.0	6.8	27.6	23.7	17.9	15.3	16.0	24.3	27.5	9.6	11.4	6.1	7.2
H	24.6	7.7	-	-	9.1	21.3	11.6	5.7	5.2	3.1	14.7	14.2	7.3	21.3	35.1	51.9
I	1.3	-	-	-	-	-	-	1.4	0.9	-	-	-	2.8	-	-	-
J	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
K	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L	2.3	-	-	-	-	-	-	-	-	-	1.2	1.1	-	1.7	2.6	-
M	3.2	1.6	21.2	6.2	3.1	4.0	9.0	1.5	1.0	-	5.7	2.6	2.0	2.7	1.8	9.9
N	4.7	3.7	12.8	3.5	7.1	5.0	8.4	0.7	0.8	-	5.1	1.4	2.1	7.7	5.8	10.5
O	2.0	1.1	3.3	-	0.6	1.1	1.8	-	-	-	1.1	-	0.5	7.7	1.0	-
P	6.2	2.1	-	-	1.2	1.7	2.5	-	0.7	-	1.5	0.9	0.8	2.4	7.8	6.4
Q	1.3	-	-	-	-	-	-	2.8	2.4	5.7	-	-	3.1	-	-	-
R	1.2	-	-	-	1.6	0.8	-	1.8	2.5	8.2	0.6	0.7	1.3	0.6	-	-
S	0.6	-	-	-	-	-	-	-	-	-	1.3	0.6	-	3.0	-	-

Table 3. Elution positions (glucose units) of standard pyridylamino-oligosaccharides on an octadecyl-silica h.p.l.c. column (Tomiya *et al.*, 1988)

No.	Pyridylamino-oligosaccharide	Mobility (no. of glucose units)
110.1	$\begin{array}{c} \text{GN}(\beta 1 \rightarrow 2)\text{M}(\alpha 1 \rightarrow 6) \\ \text{M}(\alpha 1 \rightarrow 3) \end{array} \text{M}(\beta 1 \rightarrow 4)\text{GN}(\beta 1 \rightarrow 4)\text{GN} \begin{array}{c} \text{F}(\alpha 1 \rightarrow 6) \\ \text{GN} \end{array}$	12.7
110.2	$\begin{array}{c} \text{GN}(\beta 1 \rightarrow 2)\text{M}(\alpha 1 \rightarrow 6) \\ \text{M}(\alpha 1 \rightarrow 3) \end{array} \text{M}(\beta 1 \rightarrow 4)\text{GN}(\beta 1 \rightarrow 4)\text{GN} \begin{array}{c} \text{F}(\alpha 1 \rightarrow 6) \\ \text{GN} \end{array}$	10.2
110.3	$\begin{array}{c} \text{GN}(\beta 1 \rightarrow 2)\text{M}(\alpha 1 \rightarrow 6) \\ \text{G}(\beta 1 \rightarrow 4)\text{GN}(\beta 1 \rightarrow 2)\text{M}(\alpha 1 \rightarrow 3) \end{array} \text{M}(\beta 1 \rightarrow 4)\text{GN}(\beta 1 \rightarrow 4)\text{GN} \begin{array}{c} \text{F}(\alpha 1 \rightarrow 6) \\ \text{GN} \end{array}$	13.6
110.4	$\begin{array}{c} \text{GN}(\beta 1 \rightarrow 2)\text{M}(\alpha 1 \rightarrow 6) \\ \text{G}(\beta 1 \rightarrow 4)\text{GN}(\beta 1 \rightarrow 2)\text{M}(\alpha 1 \rightarrow 3) \end{array} \text{M}(\beta 1 \rightarrow 4)\text{GN}(\beta 1 \rightarrow 4)\text{GN} \begin{array}{c} \text{F}(\alpha 1 \rightarrow 6) \\ \text{GN} \end{array}$	10.9
100.3	$\begin{array}{c} \text{GN}(\beta 1 \rightarrow 2)\text{M}(\alpha 1 \rightarrow 6) \\ \text{G}(\beta 1 \rightarrow 4)\text{GN}(\beta 1 \rightarrow 2)\text{M}(\alpha 1 \rightarrow 3) \end{array} \text{M}(\beta 1 \rightarrow 4)\text{GN}(\beta 1 \rightarrow 4)\text{GN}$	9.9
100.4	$\begin{array}{c} \text{GN}(\beta 1 \rightarrow 2)\text{M}(\alpha 1 \rightarrow 6) \\ \text{G}(\beta 1 \rightarrow 4)\text{GN}(\beta 1 \rightarrow 2)\text{M}(\alpha 1 \rightarrow 3) \end{array} \text{M}(\beta 1 \rightarrow 4)\text{GN}(\beta 1 \rightarrow 4)\text{GN}$	8.0

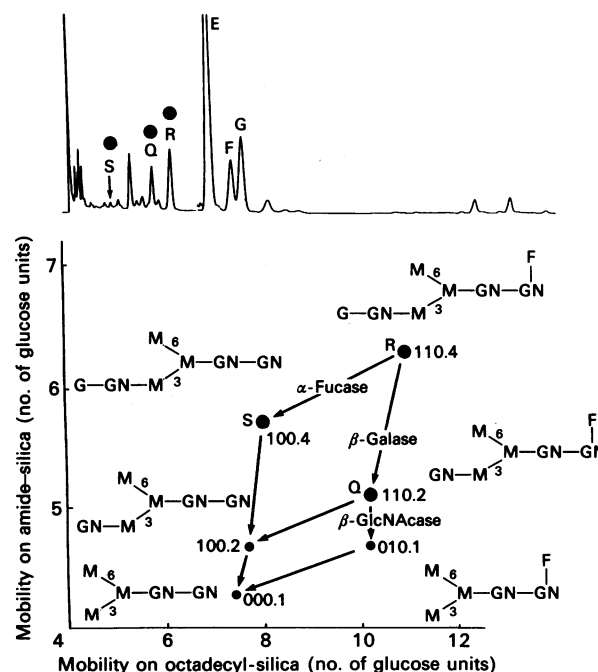
is absent from both arms. If in an intact molecule these latter (38 %) oligosaccharides were each paired with a heavy chain bearing galactose on both arms, this would account for 76 % of IgG molecules; the remaining 24 % of IgG molecules would have paired oligosaccharide moieties each having galactose on both the Man($\alpha 1 \rightarrow 3$) and Man($\alpha 1 \rightarrow 6$) arms. This provides a minimum value for the proportion of molecules bearing fully galactosylated oligosaccharide structures.

Oligosaccharides that were clearly separated on octadecyl-silica columns for each subclass sample were collected separately. Each subclass IgG oligosaccharide fraction and the corresponding authentic polyclonal IgG oligosaccharide fraction were always processed at the same time and in the same manner, and h.p.l.c. profiles were compared as described below. They were subjected to size fractionation on an amide-silica column. Each set of elution positions on octadecyl-silica and amide-silica columns were plotted on the two-dimensional sugar map prepared with 113 different oligosaccharide standards (Tomiya *et al.*, 1988). The elution positions of oligosaccharides A-P derived from the monoclonal proteins corresponded to authentic oligosaccharides A-P derived from polyclonal IgG, thus allowing unequivocal structural assignment. Indeed, ^1H -n.m.r.-spectral data for the H-1 and acetyl groups clearly indicate that oligosaccharides E, F+G, H and M-P in protein Yot and oligosaccharides E and M-P in proteins Ogo and Ike-N were demonstrated to give chemical shifts that are consistent with the proposed structures (Takahashi *et al.*, 1987).

Structure of oligosaccharides Q, R and S

Although only very small amounts of the unusual oligosaccharides Q, R and S are present in polyclonal IgG, they are clearly present in IgG2 subclass proteins T.L., Ku-3, INK and M3 and also in IgG3 subclass proteins L.T., C.H., Jir and Gal (Figs. 3 and 4). We prepared the standard oligosaccharides nos. 110.1, 110.2, 110.3, 110.4, 100.3 and 100.4 (Table 3) for determining the structure of oligosaccharides Q, R and S, as described in the Materials and methods section. Oligosaccharides Q, R and S and the standard oligosaccharides were always processed at the same time and in the same manner, and their h.p.l.c. profiles were compared. The elution position (glucose units) on an octadecyl-silica column of each pair of oligosaccharides with the same

molecular size, oligosaccharides nos. 110.1 and 110.2, oligosaccharides nos. 110.3 and 110.4 and oligosaccharides nos. 100.3 and 100.4, can be used to distinguish clearly between substituted and unsubstituted Man($\alpha 1 \rightarrow 3$) residues (Table 3). The elution positions of oligosaccharides Q, R and S on an octadecyl-silica column coincided with those of standard oligosaccharides nos.

**Fig. 6.** Determination of structures of oligosaccharides Q, R and S by using the two-dimensional sugar map method

The co-ordinates of oligosaccharides Q, R and S were superimposed on the co-ordinates of the standard oligosaccharides nos. 110.2, 110.4 and 100.4 respectively on the sugar map. Arrows indicate the direction of changes of the co-ordinates of oligosaccharides after digestion by α -fucosidase (α -Fucose), β -galactosidase (β -Galase) and β -N-acetylhexosaminidase (β -GlcNAcase).

saccharides without galactose and having galactose on the Man(α 1 \rightarrow 6) arm only, on the Man(α 1 \rightarrow 3) arm only and on both the arms respectively. It is apparent that each paraprotein has a unique oligosaccharide profile and that the relative proportions of structures E, F, G and H differ widely. Three broad patterns of glycosylation may be discerned: (1) proteins in which structure E predominates (Ogo, Ike-N, Ku-3, M3, A.M. and Rea), (2) proteins in which structures E, F, G and H are each present in significant quantity (Yot, Dob, T.L., J.D., INK, L.T., C.H., Jir, Gal and L.H.) and (3) proteins in which structure H predominates (Hay and A.S.). These differing patterns of galactosylation presumably reflect differences in the activity of galactosyl-transferases and processing enzymes within the clones of cells synthesizing the respective proteins.

It is of interest to compare and contrast the glycosylation patterns of the IgG4 proteins Rea and A.S. (Table 2), in which components E and H predominate respectively. Analysis of Fab and Fc fragments generated from these proteins show that carbohydrate was attached within the Fc region only and will therefore be linked through asparagine-297. The sum of components D.H. and P for protein A.S. gives a total of 62% of oligosaccharide components with galactose on both the Man(α 1 \rightarrow 3) and Man(α 1 \rightarrow 6) arms. This suggests that a minimum of 24% of molecules have digalactosylated oligosaccharide moieties on both heavy chains. Analysis of rabbit Fc has suggested that such structures cannot be accommodated within the inter C₂ domain space and that one Man(α 1 \rightarrow 3) arm is always devoid of galactose (Sutton & Phillips, 1983; Rademacher & Dwek, 1983). It is possible that accommodating such structures requires a change in the protein conformation.

It is noteworthy that in polyclonal IgG, IgG1 and IgG4 proteins oligosaccharide F is always present in greater amount than oligosaccharide G. This has been interpreted to suggest that galactosylation occurs preferentially on the Man(α 1 \rightarrow 6) arm of the biantennary complex. However, in all IgG2 proteins analysed and two (out of five) IgG3 proteins oligosaccharide G predominates over oligosaccharide F. These results suggest that the galactosyltransferase transfers galactose to the *N*-acetylglucosamine attached to the Man(α 1 \rightarrow 3) residue in preference to the Man(α 1 \rightarrow 6) residue. The unusual monoantennary oligosaccharides Q, R and S, which lack *N*-acetylglucosamine bonded to the Man(α 1 \rightarrow 6) residue, were also detected in subclass 2 or 3 proteins only. These results suggest a lower activity for *N*-acetylglucosaminyltransferase in IgG2-subclass-producing and

IgG3-subclass-producing plasma cells. The presence of oligosaccharide structures Q, R and S in human IgG is demonstrated for the first time in this study.

We thank Miss Tracy Bentley for her excellent technical assistance. This work was supported in part from the Ministry of Education, Science and Culture of Japan. R.J. gratefully acknowledges the Japan Society for the Promotion of Science and The Royal Society (U.K.) for financial support for a study visit.

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Received 6 July 1989/20 November 1989; accepted 21 February 1990