The Carbohydrate Structures of a Mouse Monoclonal IgG Antibody OKT3*

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A mouse monoclonal antibody OKT3, of IgG2a isotype, was isolated from hybridoma culture fluid. Sugar analysis showed the presence of sialic acid, galactose, mannose, fucose, and N-acetylgalactosamine, i.e. sugars typical for N-glycosidically linked carbohydrate chains. The absence of N-acetylgalactosamine revealed that O-glycosidically linked carbohydrates were not present. The purified antibody was reduced, alkylated, and separated into heavy and light chains, and all carbohydrates were shown to be associated with the heavy chains. The N-linked carbohydrate chains were isolated as alditos using strong alkaline-borohydride degradation and further fractionated on a concanavalin A-Sepharose column and high performance ion exchange chromatography with pulsed amperometric detection. Structural analysis was carried out on the isolated oligosaccharide alditos by chemical analyses, fast atom bombardment mass spectrometry, and 500-MHz 1'H NMR spectroscopy. Triantennary and biantennary types of structures were found. The triantennary structures were present as trisialo and tetrasialo forms without fucose; the tetrasialo forms were shown to contain a sequence of Neu5Acα2-3Galβ1-3[Neu5Acα2-6]GlcNAcβ1-4 on one of the branches. The biantennary structures were present as completely sialylated nonfucosylated species and as asialo-, agalacto-, and partially fucosylated structures.

In humans four IgG subclasses are defined, IgG1, IgG2, IgG3, and IgG4, whereas in mice they are assigned IgG1, IgG2a, IgG2b, and IgG3. The literature data on the sugar moieties of IgG molecules available so far include characterization of human- (normal and pathological (3, 4)), rabbit- (5), bovine- (6), porcine- (7), and mouse-derived species (8). The oligosaccharides of IgG of human and rabbit origin contain a bisecting N-acetylgalactosamine residue linked to the C-4 position of the 3,6-disubstituted β-mannosyl residue, whereas oligosaccharides from bovine, porcine, and mouse IgG lack this residue. The overall form of the IgG derived oligosaccharides, based on the data published so far, is a biantennary complex-type oligosaccharide chain sialylated to a different extent, having agalacto forms and partially fucosylated at the innermost N-acetylgalactosamine residue. These IgG-derived N-linked oligosaccharides represent always an array of structurally related chains, which is referred to as heterogeneity of the carbohydrate moiety in glycoproteins (9). This phenomenon gives rise to so-called glycoforms, which are defined as glycoproteins with an identical polypeptide chain but differ with respect to glycosylation (10).

The literature data on the sugar moiety of mouse monoclonal antibodies still remain obscure (11, 12). These antibodies get currently still more applications in medicine and natural sciences, e.g. in clinical medicine for various therapeutic purposes. When introduced into the human organism, they should maintain constant concentration as long as possible. Considering this property, much attention has been paid to the survival of these compounds in the circulation and, as a consequence, to the carbohydrate structures of monoclonal antibodies used in therapy, because, according to Ashwell and Harford (13), soluble asialoglycoproteins are rapidly removed from the circulation by the galactocere specific receptor on the surface of mammalian hepatocytes.

Taking all of the above into consideration, we decided to investigate in detail the carbohydrate structures present in the mouse monoclonal antibody OKT3 of IgG2a isotype (14), specific for the CD3 antigen on human T lymphocytes. This antibody is being commonly used in clinical medicine as a prophylactic immunosuppressive agent in kidney and heart transplantations (15). Moreover, we expected to gain more insight into the known phenomenon of microheterogeneity of the sugar moiety of glycoproteins (9). In this respect, the OKT3 antibody served us as an example of a glycoprotein of hFab composition, having only one N-glycosylation site per h-subunit.
EXPERIMENTAL PROCEDURES AND RESULTS

Despite the fact that considerable amounts of data are available on the sugar moieties of IgG molecules (both of human and animal origin) in health and disease, little is known about the sugar chains of mouse monoclonal antibodies (11, 12). Therefore, we decided to investigate the oligosaccharides present in the mouse monoclonal antibody OKT3 of IgG2a isotype. Two objectives of this work were considered. The first was to perform a detailed structural analysis of the oligosaccharides present in the mouse monoclonal IgG antibody, a work that has not been done so far, and the second was to establish the range of heterogeneity, if any, within oligosaccharides present in this type of molecule, i.e., molecules which according to their production process (hybridoma technique) exhibit completely uniform polypeptide chains.

The OKT3 antibody was obtained as a hybridoma culture fluid and purified by ion exchange chromatography on ZetaPrep cartridges. The preliminary sugar analysis, performed on the intact purified antibody and on separated heavy and light polypeptide chains, suggested only one N-glycosylation site per heavy chain. Therefore, in most experiments the heavy chains were used, instead of the intact antibody, for isolation of the N-linked oligosaccharides. The oligosaccharides were liberated from the polypeptide backbone by use of strong alkali under reducing conditions (18). As can be seen, triantennary and biantennary oligosaccharides were found (Fig. 6), the former being the type of structures not identified in human (3, 4), rabbit (5), bovine (6), porcine (7) and mouse (8) serum-derived IgG molecules. Moreover, the triantennary oligosaccharides were not fucosylated. All triantennary oligosaccharides were not fucosylated. All triantennary and biantennary oligosaccharides were not fucosylated. All triantennary and biantennary oligosaccharides were not fucosylated.

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A striking feature of these oligosaccharides is that only the smallest structures, not sialylated, were substituted by fucose (Fig. 6). In a recently published paper some chemical and structural analyses were performed on the sugar moiety of immunoglobulin G, secreted by 10 different murine hybridomas (11). The immunoglobulins were digested with Pronase, and the resulting glycopeptides were analyzed for the affinity to ConA. The results revealed the presence of non-reactive, weakly reactive, and strongly reactive glycopeptides which corresponds, in general, to our result obtained by using the same lectin (Fig. 2). The only completely estimated oligosaccharide structure, recognized as the predominantly expressed one for all clones studied, was the same as found by us in fraction B2 (Fig. 6).

As can be seen from the fast atom bombardment-mass spectrometry data obtained for fraction B1 (Fig. 4), two ions related to each other of m/z 668 and m/z 636 (the difference of 32 mass units indicates elimination of methanol) are present. These ions suggest the presence of a Hex-HexNAc-1 sequence. In this case, according to sugar and methylation analysis, the sequence is expected to be Gal1-3Gal1-4GlcNAcβ1-. According to Galili et al. (29) this sugar sequence exists on different cells of nonprimate mammals, prosimians, and New World monkeys and can be detected by specific antibodies. Oligosaccharide structures, terminating with this sequence, have been described in different glycoproteins and glycolipids (30). Interestingly, it was estimated that about 1% of circulating IgG antibodies in humans react with terminal α-galactose residues (29); nevertheless, the biological significance of the presence of the Gal1-3Gal1-4GlcNAcβ1- sequence in oligosaccharides has not been established yet. For the OKT3 antibody studied here, the Gal1-3Gal1-4GlcNAc sequence is at most a very minor component amounting to significantly less than 1% of the total carbohydrate. It is further likely that OKT3 molecules bearing this sequence represent only a minor species in the total preparation. According to additional experiments (data not shown), an oligosaccharide having this sequence, detected in B1 fraction, should be eluted from HPIEC in the range between subfractions B1.1 and B1.2 (Fig. 8). It should be noted that more certain proof of the existence of this linkage would require NMR analysis.

Finally, in the course of the present investigation we got some detailed information regarding the method used for isolation of the N-linked oligosaccharides from OKT3 antibody, i.e. strong alkaline degradation under reductive conditions (18). We have confirmed that this chemical method of degradation does not give a 100% yield of free reduced oligosaccharides. Some of the carbohydrate chains were obtained in the form of glycopeptides, which were easily recognized by the methylation analysis and 1H NMR spectroscopy. A more interesting feature of the method, found here, was connected with the fate of the N-acetylglucosamine residue adjacent to the peptide. We got evidence, both by 1H NMR and sugar analysis (gas/liquid chromatography-mass spectrometry), that this residue undergoes, to a certain extent, epimerization which results in a formation of mannosaminitol (Fig. 7). In conclusion, it can be stated that these two features of the strong alkaline degradation do not influence the course of structural analysis of oligosaccharide alditol itself but are drawbacks in studies of the microheterogeneity. There are a number of endoglycosidases available to release N-linked glycans. The specificity of these enzymes has recently been reviewed by Maley et al. (31).

1 Portions of this paper (including "Experimental Procedures," "Results," Tables I-IV, and Figs. 1-9) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

2 The abbreviations used are: ConA, concanavalin A; GLC-MS, gas/liquid chromatography-mass spectrometry; FAB-MS, fast atom bombardment-mass spectrometry; HPIEC-PAD, high performance ion exchange chromatography with pulsed amperometric detection; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; h, l, heavy and light polypeptide chains; Hex, hexose.
Using these enzymes would be a more attractive approach in future studies on microheterogeneity of N-linked glycans.

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high performance ion exchange chromatography with pulsed amperometric detection (HPIC-PAD). Fractions from the ConA-Sepharose column were subjected to HPIC-PAD chromatography. The system used for the analysis consisted of a Dionex Bio LC gradient pump and Bioscan PAD 2 detector. The Dionex Bio LC Module was used to sample and prepare the eluants with helium. The oligosaccharides were monitored on a Dionex CarboPak PA1 column (4.6 x 250 mm), using a flow rate of 0.4 ml/min in a gradient elution scheme. Detection of oligosaccharides was achieved with a gradient of sodium acetate in 0.2 M, starting at 50 mm sodium acetate and changing to 200 mm within 90 min. The following pulse potentials and durations were used for detection: E1 = 30 V (t1 = 30 s); E2 = 600 V (t2 = 120 s); E3 = 600 V (t3 = 420 s).

Analytical methods. - Oligosaccharides were determined by one-dimensional chromatography (ODC) as alditol acetates (13) after hydrolysis in 4 M trifluoroacetic acid at 100°C for 4 h. Alcoholic acid was determined by a colorimetric method (22). Metabolism of oligosaccharides was monitored by electrophoresis using urea polyacrylamide gels. The separation of oligosaccharides was electrophoresed in the gel mixture for 1.5 h at 300 V. Separations were performed on capillary columns 0.75 mm x 12 m for alditol acetate and 0.75 mm x 30 m for partially methylated alditol acetate.

Spectroscopic methods. - One-dimensional chromatography mass spectrometry (ODC-MS), in electron ionization mode, was carried out on a VG 72-225 quadrupole instrument fitted with a 70 eV capillary column. Spectra were recorded at 70 eV with an ion source temperature of 200°C. For fast atom bombardment mass spectrometry (FAB-MS), in positive ion mode, a VG ZAB 25 instrument was used with chloroform (1-chloro-2,3-propenylidene) as the matrix and xenon with a kinetic energy of 8 eV. 1H NMR spectra were recorded at 270 MHz with a Bruker AM-500 P.T.E. spectrometer. The oligosaccharides and glycopeptides were repeatedly exchanged with H2O and finally dissolved in a high purity (99.95%, H2O). The chemical shifts were expressed relative to internal sodium 4,4-dimethyl-4-silapentane sulfonate (DSS), but were actually measured relative to internal aqueous, set to 2.25 ppm, or to internal free aqueous, set to 1.908 ppm, with an accuracy of ±0.002 ppm.

RESULTS

Purification and analysis of the GMT antibody. - The GMT antibody was obtained as a hydroxide buffer and purified using ion-exchange chromatography on QM and SP columns. After dialysis and freeze-drying, the antibody was analyzed for sugar content. The analysis showed monosaccharides typical for N-linked sugar chains, i.e., mannose, galactose, N-acetylglucosamine, fucose, and sialic acid (Table I). The total sugar content was about 6.4% by mass. Considering N, the antibody equaled to 1.5 x 107, this result suggested about two glycosylation sites per molecule. Subsequently, the antibody was degraded and the heavy and light chains were separated. Sugar analysis of the isolated chains revealed the presence of carbohydrates exclusively in the heavy chains. This result, together with the total sugar content of the antibody, amounting to one N-glycosylation site per heavy chain in the GMT antibody molecule.

**TABLE I**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Component</th>
<th>Molar Mass</th>
<th>Relative Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PTG</td>
<td>2.6</td>
<td>0.8</td>
</tr>
<tr>
<td>2</td>
<td>MN</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>3</td>
<td>Gal</td>
<td>1.9</td>
<td>1.7</td>
</tr>
<tr>
<td>4</td>
<td>GlcNAc</td>
<td>4.5</td>
<td>4.2</td>
</tr>
<tr>
<td>5</td>
<td>NeuNAc</td>
<td>1.9</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Component | No. GalNAc or Gal was detected

Isolation and fractionation of the N-linked oligosaccharides. - The scheme of experiments used in this study for the degradation of the IgP antibody and subsequent separation of the carbohydrate chains are summarized in Fig. 1. Overlapping retention of the glycopeptide mixtures under conditions designed for liberation of the N-linked oligosaccharides (14), fractionation on a Sephadex G-15 column, re-precipitation and desalting on the same G-15 column, the mixture of oligosaccharides was fractionated on a ConA-Sepharose column (Fig. 2). Three fractions were obtained: an unbound (fraction A), a fraction eluted with 1 M methyl-mannoside (fraction B), and a fraction eluted with 200 mM methyl-mannoside (fraction C). These three fractions were purified from low molecular weight material (methyl-mannoside and salting on a Bio-Gel P-6 column). Under desalting, fraction B was eluted from the column as a single peak, whereas fractions A and C gave two peaks each, A1 and A2, C1 and C2, respectively (Fig. 3). The completeness of liberation of N-linked oligosaccharides was confirmed by analysis of the HPIC column. Two fractions were subjected to structural analysis as described below.

![](image-url) Fig. 1. Schematic procedure for the isolation and purification of the N-linked oligosaccharides from GMT antibody. The details are given in EXPERIMENTAL PROCEDURES.

Analysis of fraction A. - The elution profile of fraction A from the ConA-Sepharose column showed the presence of tri- and tetra-antennary complex type of oligosaccharides (Fig. 2). Similar analysis of this fraction showed monosaccharides commonly found in complex type of structures, without fucose (Table 1). The acetylation analysis showed mannose derivatives (1,4,6-O-Mann, 1,3,4,6-O-Mann, 1,6-O-Mann, and 1,3,4,6-O-Mann) typical for a triantennary type of structure (Table 1). The acetylation analysis also indicated that all the oligosaccharides contained internal -1,3-linked galactose residues indicated presence of 2-3 and 2-4 linked sialic acid. The major GlcNAc derivative (1,3,4-O-GlcNAc(Man)140) represented the substituted residues, but also minor amounts of other sialic derivatives were found. Fraction A, which accounted for 65.8% of the total weight of oligosaccharides, was analyzed by FAB-MS after permethylation. The primary and secondary fragments of m/z 276, 364 and m/z 823, 973 determine the substitution of GlcNAc residues at C4 position (24). In the high mass scan two prominent molecular ions (M+17 at m/z 3599 and M+29 at m/z 3629) can be seen. They are consistent with triantennary type of structures containing three respective four sialic acid residues. Fraction A was therefore further characterized on the HPIC-PAD system and subfractions A1 to A4 were collected (Fig. 3). These subfractions were further analyzed using the HPIC-PAD system.

Analysis of subfractions A1 to A4. - In-HPLC data on N-linked oligosaccharides and glycopeptides have been reviewed (25) and recently chemical shifts data on oligosaccharides from Persepolis plants were presented (25). These data were used in this work as reference data. Separation of the oligosaccharide alditol acetates present in fraction A gave four major subfractions A1, A2, A4, and A7, which were subsequently analyzed by NMR (25). Structural type of structures was requested from the FAB-MS data (Fig. 4). The chemical shifts for M+1 and M+2 of the mannose residues, together with the chemical shift values of the N-acetyl GlcNAc signals, supported this suggestion (Table II).

![image-url] Fig. 2. Elution profile of affinity chromatography on a ConA-Sepharose column. The N-linked oligosaccharides (A) were separated by semipreparative affinity chromatography of the GMT antibody. Three fractions were subjected to structural analysis as described below.
Immunoglobulin G Carbohydrates

Table II

<table>
<thead>
<tr>
<th>Sugar derivative</th>
<th>A</th>
<th>B1</th>
<th>B2</th>
<th>C1</th>
<th>C2</th>
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</thead>
<tbody>
<tr>
<td>2,3,4-O-Me-Fuc</td>
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<td>0.7</td>
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<tr>
<td>2,3,4,6-D-NeuAc</td>
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</tr>
<tr>
<td>3,4,6-D-NeuAc</td>
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<td>1.1</td>
<td>1.5</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td>2,4,6-D-NeuAc</td>
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<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>2,3,4,6-D-NeuAc</td>
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<td>0.6</td>
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<td>0.6</td>
</tr>
<tr>
<td>2,4,6,3,4,6-NeuAc</td>
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<td>0</td>
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</tr>
<tr>
<td>2,3,5,6-D-NeuAc</td>
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<td>1.0</td>
</tr>
<tr>
<td>2,3,4,5,6,7-NeuAc</td>
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<td>0</td>
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<tr>
<td>3,4,6,7-NeuAc</td>
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</tr>
</tbody>
</table>

Fig. 5. HPSEC-PAD chromatogram of fraction A. Fraction A (4.1 mg) was applied in two portions and subfractions A1 to A8 were collected as indicated.

The sequence corresponding to the major compound identified in the subfraction A2 is shown in Fig. 4.

The 1H-NMR data for the major component in subfraction A3 revealed a similar structure as in subfraction A2 with an exception of the branching position of the NeuAc residue. The ratio of NeuAc to NeuAc-9 linked NeuAc was 2:1, determined as discussed above. The chemical shifts for the amide protons of the Gal-4 at 4.44 ppm and the NeuAc at 5.133 ppm, and the MeOAc CH2 signal of GlcNAc at 2.068 ppm show that the NeuAc-linked NeuAc is attached to C-2 position of the 2,4-di-substituted mannose residue. Consequently, the NeuAc linked NeuAc must be or the other two branches, which is supported by the signals for H-1 of the Gal-6, Gal-4 and Man-6 residues. The structure corresponding to oligosaccharide eluted present as the main component in subfraction A3, as shown in Fig. 4. N-AC-MS analysis of fraction A indicated, as discussed above, the presence of tetra-substituted trisaccharide type of structure. The major components in subfractions A2 and A3 gave 1H-NMR data in agreement with data reported lately on this type of structures (76). Signals for H-1 of the Gal-4 and the NeuAc at 4.205 ppm and 5.113 ppm, respectively, together with the NeuAc CH2 signals of NeuAc-9 at 2.068 ppm and 2.070 ppm, found in the spectra of A2 and A3, show that a sequence of NeuAc(2-NeuAc)[Gal](3-Gal) is linked to C-4 position of the 2,4-di-substituted mannose residue (Table II). In subfraction A4 the other bands are tentatively identified by 2D-NMR linked NeuAc residues, inferred from the signals for H-1 of NeuAc at 4.205 ppm and 4.208 ppm, respectively. The only difference between A2 and A3 is the exchange of NeuAc linked NeuAc-9 to galactose in A3 for NeuAc linked NeuAc-9 to galactose in A2, on the branch linked to the Man-6 residue (Fig. 4). This exchange is corroborated by the shifts

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Retention time (min)

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Retention time (min)
for N-1 of Man-4 from 4.920 ppm to 4.944 ppm and the shift of N-acetyl Cya signal of GlcNAc-5 from 2.065 ppm to 2.026 ppm. All the chemical shifts, measured for the major aldolics present in subfractions A2, A3, A4 and A5 were in good agreement with those reported for oligosaccharides from bovine feces (46). In-HMQC analysis of subfraction A4 showed chemical shifts for all structural resonances except those of the major componcy in subfraction A5, except for signals of the reduced GlcNAc residue. The N-1 signal at 4.239 ppm and the N-acetyl Cya signal at 2.056 ppm were not detected in subfraction A5, as no N-acetyl Cya signal appeared at 2.036 ppm (Fig. 1). The same observation was made when comparing subfractions A1 and A6. The suggestion, inferred from these data, about a probable reduction of the carbohydrate moiety in the GlcNAc residue was confirmed by GC-MS of the super analysis sample of subfraction B5 (see below).

Therefore, subfraction B1.5 was subjected to sugar analysis, performed using GLC-MS after NaBH4 reduction of the hydrolyzed sample. A component corresponding to Gal-Man, reduced 2-acetamido-2-deoxygalactose, was detected. This result suggested that the reduced terminal had undergone modification during reductive alkaline degradation, from GlcNAc residue to Man-Manol. For analysis of the reduced 2-acetamido-2-deoxygalactose (2AcGal-Manol) at subfraction A5, due to the presence of a large number of signals, only 2-acetamido Cya signals could be detected, indicating the absence of 2-acetamido Cya signals at subfraction A1. 2-acetamido Man-Manol at subfractions B1.6 and B1.7 were found to be glycopolypeptides with variable length of the peptide moiety. The 1H-NNMR data of subfraction B1.6 showed the same carbohydrate structure as in fraction B2 (see below). Subfractions B1.6 and B1.7 were 1H-NNMR spectra due to heterogeneity of the poly saccharide portion. Nevertheless, the carbohydrate signals tended to fit to the structure included in B1 fraction, but without focus.

Analysis of fraction B2 - Fraction B2 (11.1% of the total weight of oligosaccharides), derived from fraction B under desalting, was eluted from the Bio-Gel P-4 column after fraction B1. Analysis of fraction B2 showed about one galactose residue per three fucose residue and the absence of galactose residue (Table II). A repetitive structure of the carbohydrate chain was observed for the fraction B2. The 1H-NNMR spectrum of fraction B2 is shown in Fig. 4. A sequence of GlcNAc-GlcNAc in the primary and secondary sequence (five of n = 260 and n = 278) for non-reducing terminal A-acetyl-GlcNAc indicated complete substitution of the saccharide by fucose at C-1 position. The mass of the molecule was determined by ESI mass spectrometry and found to be in agreement with the carbohydrate structure included in B1 fraction. The chemical shifts of the reduced oligosaccharides, present in fraction B2, are in agreement with the data of Antilla et al. (11), who isolated the same oligosaccharide, as the most abundant sugar structure of Immunoglobulin G secreted by murine lymphoma, in the form of a glycopeptide.

Analysis of fractions C1 and C2 - Fraction C was eluted from the Con-A-Sepharose column with 200 mM methylmannoside and gave under desalting on Bio-Gel P-4 two separated peaks C1 and C2. Fraction C1 (11.7% of the total weight of oligosaccharides), was eluted before fraction C2 and should therefore be of higher molecular weight than fraction C2. Sugar analysis of fraction C1 showed the presence of mannose, N-acetylgalactosamine and little amount of fucose (Table I). The methylation analysis showed a substituted and terminal, non-reducing N-acetylgalactosamine and two mannose derivatives 13,16,1-O-Man-Man and 2,3,4,6-Man-Man, indicating a triantennary type of structure. Analysis of fraction C2 revealed the presence of a glycopolypeptide, showing the N-1 signal of GlcNAc at about 5.05 ppm and N-acetyl Cya signal at 2.077 ppm, together with a typical branched pattern of N-1 and N-2 of the mannose residues. In this case, signals were detectable and the GlcNAc GlcNAc at about 5.05 ppm and N-acetyl Cya signal at 2.077 ppm, together with a typical branched pattern of N-1 and N-2 of the mannose residues. In this case, signals were detectable and the GlcNAc GlcNAc at about 5.05 ppm and N-acetyl Cya signal at 2.077 ppm, together with a typical branched pattern of N-1 and N-2 of the mannose residues.
Immunoglobulin G Carbohydrates

The above data indicate the presence of sialic acid and a single, homogeneously interconvertible type of structure. Fraction C2 was further investigated by 1H-NMR spectroscopy and the chemical shifts measured are shown in Table IV. The chemical shifts for H-1 and H-2 of mannose residues confirmed the presence of an iotaenne type of structure. The oligosaccharide in fraction C2 was in a form of adduct, approximately 15% of the reduced terminal was in the form of ManNAc-

Complete substitution of O-4 position of the reduced sugar by glucose was evident from GlcNAc-2H signal at 2.21 ppm and anomeric signal at 4.696 ppm. Otherwise the structure was identical to oligosaccharide in fraction C1. The structure corresponding to the oligosaccharide adduct present in fraction C1 is shown in Fig. 9.

**Fig. 7** 1H-NMR spectra (psyrrenyl Oβ region) of subfractions A3 and A4. The major oligosaccharide components in subfractions A3 and A4 are the same. The major component of subfraction A3 (lower trace) shows GlcNAc-2H configuration of the reduced terminal, δ (CH) = 2.056 ppm, whereas the component in the subfraction A4 (upper trace) shows ManNAc-2H configuration of the reduced terminal, δ (CH) = 2.33 ppm. Subfraction A3 contains also 33% of the structure present in subfraction A4, having ManNAc-2H configuration of the reduced terminal.

TABLE III

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**TABLE IV**

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**Fig. 8** APDC-PAD chromatograph of fraction B1. Separation of fraction B1 (1.0 mg) into subfractions B1.1 to B1.7.

**Fig. 9** Schematic numbering of the monosaccharide residues in the oligosaccharide adducts from the W3 antibody (see Tables II and IV).
The carbohydrate structures of a mouse monoclonal IgG antibody OKT3.
H Krotkiewski, G Grönberg, B Krotkiewska, B Nilsson and S Svensson

J. Biol. Chem. 1990, 265:20195-20201.

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