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 alditols 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 alditols by chemical analyses, fast atom bombardment mass spectrometry, and 500-MHz 1H 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 Neu5Acα2-3Galβ1-4GlcNAcβ1- on one of the branches. The biantennary structures were present as completely sialylated nonfucosylated species and as asialo-, agalacto-, and partially fucosylated structures.

Immunoglobulin G antibodies, both of human and animal origin, have been the subject of numerous papers dealing with physical, chemical, and immunological properties. The IgG molecules have a molecular mass of 150 kDa and consist of two heavy and two light polypeptide chains (h\textsubscript{L}). It is well established that normal IgG class antibodies have one conserved N-glycosylation site at Asn-297 in the C\textsubscript{L}2 domain of the Fc region of each heavy chain. Some other glycosylation sites, nonconserved and randomly occurring, are found in the Fab region (1). Therefore, the average number of oligosaccharide chains in an intact IgG molecule was estimated to be 2.8

(2). 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 moieties 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 galactose 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 h\textsubscript{L} composition, having only one N-glycosylation site per h-subunit.

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20195
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, still 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 Zeta-Prep 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 facsyolated. All triantennary oligosaccharides, eluted as an unbound fraction from a ConA-Sepharose column, were completely sialylated, and the type of linkage of Neu5Ac residues was responsible for the differences between these oligosaccharides. An interesting feature of two of these triantennary oligosaccharides was a feature of two of these triantennary oligosaccharides was a sequence containing a disialylated type I chain Neu5Accr2-ac2-6GlcNAc1 (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 Hexa-HexNAc1sequence. In this case, according to sugar and methylation analysis, the sequence is expected to be Gal1-3Gal1-4GlcNAc1. 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 Gal1-3Gal1-4GlcNAc1sequence 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 fractions 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 mannosaminotol (Fig. 7).

Moreover, the oligosaccharide aldolites having the same total structure except reduced terminal (in the form of N-acetylglucosaminotol or N-acetylmannosaminotol) can be separated on the HPIEC system (Figs. 5 and 8). 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 aldolite 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, 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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REFERENCES


Supplementary Materials

Isolation of the heavy and light polypeptide chains. — The purified antibody was extensively reduced and alkylated as previously described (17). The heavy and light chains were separated on a Sephacryl S-100 column (2.5 x 144 cm), eluted with 0.01 M sodium phosphate buffer at 4°C. The separated chains were extensively dialyzed against 50 mM sodium phosphate buffer, pH 7.0, and stored at -20°C.

Isolation of the N-linked glycans. — The N-linked oligosaccharides were liberated as alditols under strong alkaline conditions as previously described (16). Briefly, a 0.1% solution of the antibody (50 mg in 2 ml) was incubated at 100°C for 4 hr. The solution was cooled, neutralized with 50% aqueous acetic acid, centrifuged, and applied to a Sepharose 4B column (3 x 64 cm) eluted with 50 mM sodium phosphate. The effluent from the column was monitored by refractive index. The super-conducting fraction was recovered, de-N-acetylated with 2% acetic anhydride in ethanol (12) and desalted on the same column eluted with water.

Coosulfarla A (ConA)-Sepharose affinity chromatography. — The fraction containing the N-linked oligosaccharide molecules bound to ConA-Sepharose was desalted and neutralized to pH 7.0. The fraction was then applied to a 5 ml column of ConA-Sepharose and eluted with a methyl-β-mannoside concentration greater than 200 mM. The effluent from the column was monitored by the absorbance at 206 nm. The recovery from the ConA column was routinely 90–95% based on the sugar content. The super-conducting fractions were desalted on a Bio-Gel P-6 (200–400 mesh) column (1 x 44 cm) eluted with water.
Immunoglobulin G Carbohydrates

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High performance ion exchange chromatography with pulsed amperometric detection (HPIC-PAD). Fractions from the Con A-Sepharose column were subjected to HPIC-PAD chromatography. The system used for the analysis consisted of a Dionex Bio LC gradient pump and micro PAD 2 detector. The Dionex Variant Vegas Module was used to suspend and preserve the eluates with helium. The oligosaccharides were monitored on a Phenomenex Partisil 5 column (4.6 x 300 mm), using a flow rate of 1 ml/min of gradient elution. The separation of oligosaccharides detected was achieved with a linear gradient of sodium acetate in 0.2 M NaOH, starting with 50 mM sodium acetate and changing to 300 mM in 90 min. The following pulse potentials and durations were used for detection: E1 = 300 mV (1 min); E2 = 800 mV (15 min); E3 = 600 mV (22 min). M2 = 420 mV.

Analytical methods. Monosaccharides were determined by gas-liquid chromatography (GLC) as alditol acetates (13) after hydrolysis in 4 M trifluoroacetic acid at 100°C for 4 h, alditol acids were determined by a colorimetric method (22). Methylation of oligosaccharides and monosaccharides was carried out at 65°C using trimethylsilylation reagents (14). Methylation products were purified using Sep-Pak preparative phase cartridges (22) and converted into partially methylated alditol acetates (23) using the same conditions for hydrolysis as above. For GLC, a Hewlett- Packard 5890 instrument was used, equipped with a flame ionization detector. Separations were performed on capillary columns DB-225 (0.25 mm x 12 m) for alditol acetates and 5% -30 (0.25 mm x 50 m) for partially methylated alditol acetates.

Spectroscopic methods. The gas-liquid chromatography mass spectrometry (GLC-MS) in electron ionization mode, was carried out on a VG 12-225 quadrupole instrument fitted with an ECD-50 capillary column (0.25 mm x 30 m). 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 5F instrument was used with glycolipid (1-thio-2,3-O-propylidene) as the matrix and xenon as a kinetic energy of 40 eV. FAB-MS spectra were recorded at 27°C with a Bruker Auto MS 7.0 spectrometer. The oligosaccharides and glycoproteins were repeatedly exchanged with MeOH and finally dissolved in a high purity (99.99%) H2O. The chemical shifts were expressed relative to internal 4,4-dimethyl-4-silapentane sulfonic acid (DSS), but were actually measured relative to internal acetone, set to 2.235 ppm, or internal free acetone, set to 1.93 ppm, with an accuracy of ±0.002 ppm.

RESULTS

Purification and analysis of the OMT antibody. - The OMT antibody was obtained as a hyperimmune rabbit fluid and purified using ion-exchange chromatography on QAE and SP Sepharose. After dialysis and freeze-drying, the antibody was analyzed for sugar moieties. The analysis showed no monosaccharides typical for α-linked sugar chains, i.e., mannose, galactose, N-acetylgalactosamine, fucose and sialic acid (Table I). The total sugar content was about 6.4% by weight. Considering λ2 of the antibody equal to 1.5 x 105, 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, suggested one αglycosylation site per heavy chain in the OMT antibody molecule.

TABLE I

MONOSACCHARIDE COMPOSITION OF α-CON A AND CON A-Sepharose - derived fractions, after desialation.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Form</th>
<th>Mass</th>
<th>Relative mass</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrulose</td>
<td>0.8</td>
<td>0</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Gal</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>GalNAc</td>
<td>1.9</td>
<td>3.0</td>
<td>1.1</td>
<td>0</td>
</tr>
<tr>
<td>GlcNAc</td>
<td>4.5</td>
<td>5.0</td>
<td>4.2</td>
<td>4.2</td>
</tr>
<tr>
<td>N-acetyl GlcNAc</td>
<td>1.9</td>
<td>5.0</td>
<td>3.3</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Fructose No GalNAc or Glc was detected

Isolation and fractionation of the α-linked oligosaccharides. - The scheme of experiments used in this study for the degradation of the IgG antibody and subsequent separation of the carbohydrate chains are summarized in Fig. 1. Overlapping retention (Fig. 2, Preparation of monosaccharide standards for derivatization of the α-linked oligosaccharides (Fr)), fractionation on a Sephadex G-15 column, re-Acetylation and desialylation on the same G-15 column, the mixture of α-linked oligosaccharides was fractionated on a Con A-Sepharose column (Fig. 2). Three fractions were obtained: an unbound fraction (fraction A), a fraction eluted with 1 m NaCl-mannose (fraction B) and a fraction eluted with 200 mM methyl-mannoside (fraction C). These three fractions were purified from low molecular weight material (inorganic-mannoside and salt) on a Bio-Rad F-6 column. After dialysis, fraction A was eluted from the column at a single peak. Whereas fractions B and C gave two peaks each, AI and B1, C1 and C2, respectively (Fig. 3). The completeness of liberation of α-linked oligosaccharides averaged between 70% as judged from analysis of the HPIC-Com. About five fractions were subjected to structural analysis as described below.

Fig. 1. Schematic procedure for the isolation and purification of the α-linked oligosaccharides from OMT antibody. The details are given in EXPERIMENTAL PROCEDURES.

Analysis of fraction A. - The elution profile of fraction A from the Con A-Sepharose column suggested the presence of triand/or tetra-antennary complex type of oligosaccharides (Fig. 2). Data analysis of this fraction showed monosaccharides comonomers found in complex type of structures, without fucose (Table I). The methylation analysis showed mannose derivatives (1,4,4-OMe-Man, 1,6-OMe-Man and 1,4,6-OMe-Man) typical for a triantennary type of structure (Table II); α-fucosides (α-1,3-fucosyl) and α-fucopyranosyl (α-1,2-fucosyl) galactose residues indicated presence of 2-3 and 2-4 linked sialic acid. The major GlcNAc derivative (1,4-OMe-GlcNAc) represented 4-substituted residue, but also minor amounts of other GlcNAc derivatives were found. Fraction A, which accounted for 40.5% of the total weight of oligosaccharides, was analyzed by FAB-Ms after derivatization (Fig. 4). The primary and secondary fragments of m/z 276, 344 and m/z 812, 737 with fragmentation of N-acetyl-GLcNAc, where the molecular ion (m/z 359) and its fragment (m/z 260) were detected. The substitution of the GlcNAc residues at C-4 position (28). In the high mass area two prominent molecular ions (m/z 17) at m/z 359 and m/z 260 could be seen. They are consistent with triantennary type of structure containing three respective four sialic acid residues. Fraction A was therefore further characterized on the HPIC-PAD system and subfractions AI to AI were collected. (Fig. 5). These subfractions were further analyzed using HPLC-MS spectrometry.

Analysis of subfractions AI to AI. - In-HPLC data on α-linked oligosaccharides and glycopeptides have been reviewed (21) and recent chemical shifts data on oligosaccharides from previous studies have been reviewed (21). These chemical shifts were used in this work as reference data. Separation of the oligosaccharides detected in fraction A were four major subfractions A1, A2, A3 and A4, which were subsequently analyzed (Fig. 5). Spectroscopic type of structure was requested from the TAM-PAD data, (Fig. 6). The chemical shifts for H1 and H2 of the mannose residues, together with the chemical shift values of the N-acetyl GlcNAc signals, supported this suggestion (Table III).
Metathylation analysis of ConA-Sepharose - derived fractions. After resorcylation, the amount of 2,3,4-O-Me-Manc was set to 3.0; 0, identified by GLC-MSS but not quantified (trace; less than 0.01); A, not identified due to the absence of the reducing terminal disaccharide in a form of glucosamine.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>2,3,4-O-Me-Manc</th>
<th>2,3,6-O-Me-Gal</th>
<th>3,4,6-O-Me-Man</th>
<th>2,4,6-O-Me-Gal</th>
<th>3,4,6-O-Me-Gal</th>
<th>2,3,6-O-Me-Gal</th>
<th>1,3,5,6-O-GlcNAc(H)Ac</th>
<th>3,4,6-O-GlcNAc(H)Ac</th>
<th>3,6-O-GlcNAc(H)Ac</th>
<th>4,6-O-GlcNAc(H)Ac</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.0</td>
<td>0.2</td>
<td>0.8</td>
<td>0.0</td>
<td>0.2</td>
<td>0.8</td>
<td>0.0</td>
<td>0.8</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>B</td>
<td>0.0</td>
<td>0.2</td>
<td>0.8</td>
<td>0.0</td>
<td>0.2</td>
<td>0.8</td>
<td>0.0</td>
<td>0.8</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>C</td>
<td>0.0</td>
<td>0.2</td>
<td>0.8</td>
<td>0.0</td>
<td>0.2</td>
<td>0.8</td>
<td>0.0</td>
<td>0.8</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>D</td>
<td>0.0</td>
<td>0.2</td>
<td>0.8</td>
<td>0.0</td>
<td>0.2</td>
<td>0.8</td>
<td>0.0</td>
<td>0.8</td>
<td>0.2</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Based on the 1H-NMR spectrum, subfraction A2 was shown to contain two major components, having diol acid linked GlcA-1 and GlcA-2 to galactose residues in a ratio of 1:1, determined from the H-3ax and H-3eq signals at 1.270/2.670 ppm. The location of the GlcA-4 linked Neu5Ac residues was deduced from the chemical shifts for Neu5Ac-Manc and Neu5Aca-5: 5.103 ppm and 4.338 ppm, respectively, which indicated Neu5Ac linked 0H-5 to the branches converted to Man-4 and to C2 position of Man-4. Accordingly, the Man-4, GlcNAc signal of GlcNAc-7 at 7.075 ppm determined the Neu5AcM2-MancBI-

Fig. 4. PAGE-MS data on permethylated fractions after a ConA-Sepharose column and desalting.

Fig. 5. HPLC-PAD chromatogram of fraction A. Fraction A (4.1 mg) was applied in two portions and subfractions A1 to A5 were collected as indicated.

The sequence on the branch linked to C4 position of Man-4. The structure corresponding to the major compound identified in the subfraction A2 is shown in Fig. 4.

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 Neu5Ac residue. The ratio of GlcA-2 to GlcA-4 linked Neu5Ac was 2:1, determined as discussed above. The chemical shifts for the amnio group of Neu5Ac at 4.444 ppm and the Man-4 at 5.133 ppm, and the Proton C-2 signal of GlcNAc at 2.586 ppm show that the GlcA-2 linked Neu5Ac is attached to C2 position of the 2,4-di-substituted mannose residue. Conversely, the GlcA-4 linked Neu5Ac must be on the other two branches, which is supported by the signals for H-3 of the Gal-6 and Gal-4 of Man-4 residues. The structure corresponding to oligosaccharide eluted present as the main component in subfraction A3, is shown in Fig. 4. PAGE-MS analysis of fraction A indicated, as discussed above, the presence of tetrasaccharidyl tetrasaccharide type of structures. The major component in subfractions A6 and A7 gave 1H-NMR data in agreement with data reported lately on this type of structure (76). Signals for H-6 of the GlcA-4 and the Man-4 at 5.103 ppm and 5.938 ppm, respectively, together with the Man-4 C-2 signals of GlcNAc-7 at 7.075 ppm and 2.586 ppm, found in the spectra of A6 and A7 show that a sequence of Neu5Acm2-MancBI-(3)Neu5Acm2-MancBI-MancBI-(3)Neu5Acm2-MancBI linked to C4 position of the 2,4-disubstituted mannose residue (Table III). In subfraction A6 the other two branches are differentiated by GlcA-2 linked Neu5Ac residues, inferred from the signals for H-6 of the GlcA-4 and the Man-4 at 5.103 ppm and 5.938 ppm, respectively. The only difference between adducts present in subfractions A6 and A7 is the exchange of Neu5Ac linked GlcA-2 to galactose in A6 for Neu5Ac linked 0H-5, to galactose in A7, on the branch linked to the Man-4 residue (Fig. 6). This exchange is monitored by the shift
Immunoglobulin G Carbohydrates

The chemical analyses and the FAB-MS data clearly showed that fraction B1 contained several structures. Fraction B3 with therefore further characterized on the HUIC-FAB-MS system, giving information about the mass of the peaks (Fig. 1). These subfractions were further characterized by 1H-NMR spectroscopy in D2O (Fig. 2). Analysis of subfractions B1 and B7 - 1. Two major oligosaccharide sub-classes, eluted at B1 and B7.4 subfractions, were obtained from the HUIC-FAB-MS (Fig. 1). Analysis by 1H-NMR revealed the chemical shifts for H-1 and H-2 of the mannose residue, which was found to be the same in both subfractions. The peak position of the 1H-NMR spectrum shows that the peak of the major substructure is consistent with the position of the 1H-NMR signal of the main substructure.

Therefore, subfraction B1 was subjected to sugar analysis, performed using GC-MS after HPAE reduction of the hydrolyzed samples. A component corresponding to NeuAcα2→3Galβ1→4GlcNAcβ1→4Galβ1→ sequentially cleaved from the main substructure in the major substructure. The chemical shifts for H-1 and H-2 of the major substructure were consistent with the position of the 1H-NMR signal of the main substructure.

Analysis of fraction B2 - Fraction B2 (11.1% of the total weight of oligosaccharides), derived from fraction C under denaturing, was eluted from the CoA-Sepharose column with 15 mM sodium dodecyl sulfate and 10% of the sample was analyzed by 1H-NMR spectroscopy. A spectrum of the main substructure was consistent with the position of the 1H-NMR signal of the main substructure.

Analysis of fraction C1 - Fraction C1 (0.18% of the total weight of oligosaccharides), derived from fraction C under denaturing, was eluted from the CoA-Sepharose column with 15 mM sodium dodecyl sulfate and 10% of the sample was analyzed by 1H-NMR spectroscopy. A spectrum of the main substructure was consistent with the position of the 1H-NMR signal of the main substructure.
Immunoglobulin G Carbohydrates

Fig. 7 1H-NMR spectra (1H-1H 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-ol configuration of the reduced terminal; δ CH3 = 2.054 ppm, whereas the component in the subfraction A4 (upper trace) shows ManNAc-ol configuration of the reduced terminal; δ CH3 = 2.054 ppm. Subfraction A3 contains also 3% of the structure present in subfraction A4, having ManNAc-ol configuration of the reduced terminal (assignments in parentheses); * indicates deuterated signal from 1H satellite of the acetate signal (1J-H = 127 Hz), due to presence of sodium acetate.

Fig. 8 1H-NMR spectra (1H-1H 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-ol configuration of the reduced terminal; δ CH3 = 2.054 ppm, whereas the component in the subfraction A4 (upper trace) shows ManNAc-ol configuration of the reduced terminal; δ CH3 = 2.054 ppm. Subfraction A3 contains also 3% of the structure present in subfraction A4, having ManNAc-ol configuration of the reduced terminal (assignments in parentheses); * indicates deuterated signal from 1H satellite of the acetate signal (1J-H = 127 Hz), due to presence of sodium acetate.

Fig. 9 Schematic numbering of the monosaccharide residues in the oligosaccharide alditol from the W3 antisera (see Tables II and IV).
The carbohydrate structures of a mouse monoclonal IgG antibody OKT3.
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