The Oligosaccharide Units of a Human Type L Immunoglobulin M (Macroglobulin)

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1. The carbohydrate composition of the monomeric unit of a type L macroglobulin (immunoglobulin M) was determined as 6 residues of fucose, 35 of mannose, 11 of galactose, 27 of *N*-acetylglucosamine and 9 of sialic acid. 2. Two types of oligosaccharide unit were present in the protein, one of which (CA type) contained fucose, mannose, galactose, *N*-acetylglucosamine and sialic acid in the molar proportions 1:3:4:2:3–5:0–2, and the other (CB type) contained mannose and *N*-acetylglucosamine in the proportions 6–8:2–3. 3. A tentative structure is proposed for the CB type unit. 4. An *S*-carboxymethylcysteine-containing glycopeptide with a CA-type unit was isolated after reduction, alkylation and tryptic digestion of the protein. 5. The immunoglobulin monomer appears to contain six oligosaccharide units of the CA type and two of the CB type.

Human IgM* monomers (mol.wt. 180 000) contain fucose, mannose, galactose, *N*-acetylglucosamine and sialic acid in the approximate molar proportions 10:35:10:30:5 (Davie & Osterland, 1968; Spragg & Clamp, 1969). These monosaccharides are distributed between a number of oligosaccharide units, estimates of which have been made from studies on the isolated glycopeptides. Thus Bourrillon & Razafimahaleo (1967) isolated the tryptic glycopeptides of a macroglobulin and calculated that each monomer contained 10 or 12 oligosaccharide units. Spragg & Clamp (1969) isolated the Pronase glycopeptides from three type K macroglobulins, two of which contained eight and one of which contained 10 oligosaccharide units. Their results also indicated that IgM contains two types of oligosaccharide unit, one of which is similar to that found in IgG (Clamp & Putnam, 1964) and IgA (Dawson & Clamp, 1968), consisting of fucose, mannose, galactose, *N*-acetylglucosamine and sialic acid in the approximate molar proportions 1:3:2:3–4:0–2. The other type of unit, which is also found in IgA (Dawson & Clamp, 1968) and probably some IgG molecules (J. R. Clamp, unpublished work), contains only mannose and *N*-acetylglucosamine with the mannose present in amounts greater than *N*-acetylglucosamine. The macroglobulins were found to contain six of the former type of unit and from two to four of the latter (Spragg & Clamp, 1969). Similar results have

been reported by Jouanneau, Razafimahaleo & Bourrillon (1970). Davie & Osterland (1968) isolated three Pronase glycopeptides from each of two macroglobulins, and calculated that both proteins contained eight oligosaccharide units. Miller (1970) isolated glycopeptides by exclusion chromatography of trypsin and Pronase digests of macroglobulins, but the number of oligosaccharide units in the proteins was not estimated.

Beale & Buttress (1969) found four oligosaccharide attachment sites on a μ chain, three of them being located close to disulphide bridges. The fourth unit was thought to be attached within the Fd region.

No structural studies have so far been reported on the oligosaccharide units present in human IgM. In this investigation, therefore, the structures of the two types of oligosaccharide unit present in a human type L IgM have been studied. In addition, the number, composition and region of attachment of the oligosaccharide units of this protein have been investigated.

MATERIALS

All reagents were AnalaR grade where available. Carbohydrates, Pronase and urease-active jack-bean meal were obtained from BDH Chemicals Ltd., Poole, Dorset, U.K. Trypsin (type 1, 2 $\times$ recrystallized, 1000 units/mg) was obtained from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K.; 4-nitrophenyl α -D-mannopyranoside, 4-nitrophenyl *N*-acetyl- β -D-glucosaminide and 2-nitrophenyl β -D-galactopyranoside were obtained from Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.

* Abbreviations: IgM, immunoglobulin M (macroglobulin); IgG, immunoglobulin G; IgA, immunoglobulin A.

Plasma from a patient (Don), with Waldenström's macroglobulinaemia was kindly supplied by Dr J. Verrier Jones (Department of Medicine, University of Bristol).

The following buffer solutions were used: citrate buffer, pH 4.5, 0.055 M-sodium citrate; acetate buffer, pH 5.0, 0.05 M-sodium acetate containing 0.1 M-NaCl and 0.1 mM-ZnSO₄; phosphate buffer, pH 7.5, 0.02 M-Na₂HPO₄-NaH₂PO₄; tris-HCl buffer, pH 7.8, 0.05 M-tris-HCl, containing 1.0 M-NaCl; tris-HCl buffer, pH 8.0, 0.05 M-tris-HCl, containing 0.2 M-NaCl; NH₄HCO₃ buffer, pH 8.5, 0.1 M-NH₄HCO₃; borate buffer, pH 9.8, 0.2 M-sodium borate.

METHODS

Preparation of IgM. IgM was isolated by euglobulin precipitation and exclusion chromatography, and checked for purity by immunoelectrophoresis as described by Spragg & Clamp (1969).

Preparation and isolation of glycopeptide material after digestion with Pronase. IgM was digested with Pronase, and the glycopeptides were isolated by exclusion chromatography and paper chromatography as described by Spragg & Clamp (1969). The glycopeptides were finally separated by high-voltage electrophoresis in pyridine-acetic acid-water (1:10:289, by vol.), pH 3.6 (Spragg & Clamp, 1969), except that in some specified cases the duration of electrophoresis was extended to 5 h. The purity of the glycopeptides was checked by two-dimensional peptide 'mapping' as described by Clamp & Putnam (1964).

Preparation and isolation of glycopeptides after digestion with trypsin. Don IgM (500 mg) in 50 ml of tris-HCl buffer, pH 8.0, was reduced with dithiothreitol (38.6 mg) for 1 h at room temperature under an atmosphere of N₂. To this solution was then added 0.12 M-iodoacetic acid (5 ml), and a further 5 ml was added after 1 h. The reduced, alkylated protein was dialysed against three changes of NH₄HCO₃ buffer, pH 8.5. The non-diffusible material was incubated with trypsin (10 mg) at 37°C for 5 h and the solution was then freeze-dried and dissolved in a small volume of 0.15 M-NaCl. The solution was subjected to exclusion chromatography on a column (150 cm × 2.5 cm) of Sephadex G-50 equilibrated with 0.15 M-NaCl. Fractions (5 ml) were collected, and the hexose content of a sample (0.1 ml) from alternate tubes was determined by the method of Dubois, Gilles, Hamilton, Rebers & Smith (1956). The hexose-containing tubes were pooled into four fractions, which were then desalted by exclusion chromatography on a column (50 cm × 1.5 cm) of Sephadex G-10 equilibrated with water. The excluded hexose-containing fraction was freeze-dried, and the glycopeptide purified by paper chromatography and high-voltage electrophoresis (Spragg & Clamp, 1969).

Carbohydrate analyses by g.l.c. The carbohydrate content of proteins and glycopeptides, as well as the amounts of free monosaccharides, were determined by a modification (Bhatti, Chambers & Clamp, 1970) of the method of Clamp, Dawson & Hough (1967).

Amino acid analyses. Glycopeptides were hydrolysed with 6 M-HCl at 110°C for 24 h. The dried hydrolysates were then examined for their amino acid content by Dr B. T. Pickering, Department of Pharmacology, University of Bristol, or by Dr J. Williams, Department of Biochemistry, University of Bristol, by the method of Spackman, Stein & Moore (1958).

Ninhydrin assays. Exclusion-chromatography effluent fractions were assayed for ninhydrin-positive material by the method of Moore & Stein (1954).

Estimation of glycopeptide molecular weights. The molecular weights of glycopeptides were estimated by exclusion chromatography by the method of Bhatti & Clamp (1968).

Calculation of the molecular composition of glycopeptides. The equivalent weights of the glycopeptides were calculated from the aspartic acid content (Dawson & Clamp, 1968). The molecular composition of the glycopeptides was calculated by a least-squares procedure used for proteins by Katz (1968). This procedure was adapted, treating each monosaccharide residue in the same manner as an amino acid residue, and then used to determine the molecular composition and molecular weight of each glycopeptide most consistent with its composition and molecular weight as estimated by exclusion chromatography. The calculations were performed by using a computer program written in Elliott ALGOL and run on the University of Bristol Elliott 503 computer.

Glycosidase assays. The enzyme unit used is that defined by Li (1967) as the amount of enzyme liberating 1 μmol of nitrophenol from the appropriate nitrophenyl glycoside in 1 min at 25°C.

Preparation of glycosidases. α-D-Mannosidase was extracted from jack-bean meal by the procedure of Li (1967), and then purified by the method of Snaith & Levvy (1968). Mannosidase was prepared together with β-N-acetylglucosaminidase from jack-bean extracts as described by Snaith & Levvy (1968). β-N-Acetylglucosaminidase was prepared as described by Andersen (1969).

Incubation of glycopeptides with glycosidases. Glycopeptides were incubated with glycosidases for 75 h at 37°C. Samples (0.1 ml) were removed at intervals and added to an ignition tube containing arabinitol (50 nmol). The tube was heated in a boiling-water bath for 3 min to destroy enzyme activity, then dried and submitted for g.l.c. analysis of the liberated monosaccharides. To isolate the mannosidase-resistant product, the incubation mixture of the glycopeptide was concentrated under reduced pressure at 40°C. The solution was then subjected to exclusion chromatography on 0.15 M-NaCl on a column (50 cm × 1.5 cm) of Sephadex G-50. Fractions (1 ml) were collected, and the E₂₈₀ was determined. Samples (0.1 ml) were removed for determination of ninhydrin-positive material. Included fractions containing ninhydrin-positive material were pooled and concentrated under reduced pressure at 40°C, then subjected to exclusion chromatography on a column (78 cm × 1 cm) of Sephadex G-15. The effluent was monitored for ninhydrin-reacting material (0.1 ml sample) and for free monosaccharide (0.01 ml sample). The ninhydrin-positive fractions that did not contain monosaccharides were pooled.

Periodate oxidation of glycopeptides. Periodate oxidation was carried out by the procedure of Dawson & Clamp (1968). The oxidation products were desalted by exclusion chromatography on Sephadex G-10 before amino acid and carbohydrate analysis.

RESULTS

Preparations of IgM and Pronase glycopeptides. Don IgM was shown to be pure by immunoelectro-

phoresis. The protein was of antigenic type L, and contained no type K determinants, giving additional evidence of purity. Don IgM was found to contain in each monomeric unit 6 residues of fucose, 35 of mannose, 11 of galactose, 27 of *N*-acetylglucosamine and 9 of sialic acid.

Pooled glycopeptides were prepared from the macroglobulin after Pronase digestion and then separated by high-voltage electrophoresis. Electrophoresis for 5 h was found to be necessary to resolve some of the electrophoretic components, but under these conditions some glycopeptides migrated off the paper. The glycopeptides were therefore first separated into four fractions by electrophoresis for 3 h, and these fractions were then subjected to further electrophoresis for 5 h from a suitably adjusted base-line to give the individual glycopeptides (Fig. 1).

Characterization of glycopeptides. The amino acid and carbohydrate composition of the glycopeptides was determined, and their molecular weights were estimated by exclusion chromatography. The molecular compositions and corresponding molecular weights calculated by the least-squares procedure by using this data are given in Tables 1 and 2. The calculated molecular weight

corresponds to the equivalent weight, or a whole multiple thereof, in 12 of the 14 glycopeptides analysed.

Studies on half-cystine-containing glycopeptides. Tryptic glycopeptides were prepared from reduced and alkylated Don IgM. Four hexose-containing fractions were obtained by exclusion chromatography (Fig. 2). The first hexose-containing peak appeared to consist of largely undigested material, since peptide 'mapping' showed ninhydrin-positive material only at the origin. The other fractions, however, contained both glycopeptides and peptides. These fractions were subjected to descending paper chromatography in butan-1-ol-acetic acid-water (12:3:5, by vol.) to separate the peptides from the glycopeptides, which were then purified by high-voltage electrophoresis. Three glycopeptides were obtained from both the second and third fractions, and one from the fourth fraction. Two of the glycopeptides, isolated from fraction 3, had an amino acid composition similar to that of the glycopeptides containing *S*-carboxymethylcysteine isolated by Beale & Buttress (1969) (Table 3). The Don tryptic glycopeptides contained fucose, mannose, galactose, *N*-acetylglucosamine and sialic acid in the first case in the molar propor-

Fig. 1. Separation by high-voltage electrophoresis of the glycopeptides present in a Sephadex G-25 fraction from a Pronase digest of Don IgM. The glycopeptide material was applied to Whatman 3MM filter paper and subjected to electrophoresis for 3 h at 2 kV and 100 mA in pyridine-acetic acid-water (1:10:289, by vol.), pH 3.6. At the end of this time, glycopeptides 1a and 13 had migrated 22 and 13 cm respectively from the origin. The glycopeptides are numbered according to their increasing mobility towards the anode.

Table 1. *Molecular weights and compositions of type C_B glycopeptides*

The calculated molecular weight (Calc. mol.wt.) is by the least-squares procedure of Katz (1968); the estimated molecular weight (Est. mol.wt.) is by exclusion chromatography (Bhatti & Clamp, 1968) and the equivalent weight (Equiv.wt.) is from the aspartic acid content. The calculated molecular weight was used for the molecular composition of glycopeptides. The C_B glycopeptides contain only mannose and *N*-acetylglucosamine. The carbohydrate and amino acid contents of the glycopeptides were related by volume.

Residue	Glycopeptide				
	1a	1b	2	3	6
Yield (mol/100 mol of total glycopeptides)	2	2	1	6	20
Man	5.8	6.7	6.8	8.0	6.9
Gal	0	0.3	0	0	0
GlcNAc	2.3	2.7	2.1	1.7	1.9
Asp	0.9	1.1	1.2	1.3	1.1
Ser	0.1	0.1	0.6	0.5	0.3
Pro	1.0	1.1	1.7	0.3	0
Val	0	0	0.3	1.0	0
Calc. mol.wt.	1630	1900	1920	1920	1650
Equiv.wt.	1750	1760	1780	1540	1600
Est. mol.wt.	1520	1540	1540	1540	1270

Table 2. *Molecular weights and compositions of type CA glycopeptides*

Details are as described in the legend to Table 1. *N*-Acetylneuraminic acid is abbreviated to NeuNAc. CA glycopeptides contain fucose, mannose, galactose and *N*-acetylglucosamine.

Residue	Glycopeptide								
	4	5	7	8	9	10	11	12	13
Yield (mol/100 mol of total glycopeptide)	14	1	14	6	3	6	13	9	3
Fuc	0.8	0.9	1.0	0.9	1.0	0.8	0.9	0.8	0.7
Man	2.9	4.1	3.0	2.8	3.7	2.9	2.8	2.9	3.0
Gal	1.5	2.0	2.0	1.9	2.3	2.2	1.9	1.9	2.0
GlcNAc	4.1	4.9	4.9	4.2	5.0	4.0	3.3	4.2	4.1
NeuNAc	0	0	0.9	1.0	1.7	1.0	0.9	1.1	1.5
Asp	1.1	2.1	1.2	1.9	2.0	1.0	1.1	1.4	1.5
Ser	0	0.9	0.2	0	0.6	0.1	0.2	0.2	0.1
Glu	0	0.3	0.4	0	0.4	0.1	1.1	0.8	0.1
Calc. mol.wt.	1810	2460	2410	2300	2950	2200	2100	2360	2370
Equiv.wt.	1770	1260	2050	1250	1500	2170	2000	1690	1570
Est. mol.wt.	1750	1820	1910	1970	1970	1870	1970	1990	1920

Fig. 2. Chromatography on Sephadex G-50 of a tryptic digest of reduced and alkylated IgM. Samples (0.1 ml) from each tube (5 ml) were assayed for hexose content by the method of Dubois *et al.* (1956). Tubes were pooled to give four fractions as follows: fraction 1, tubes 28–39; fraction 2, tubes 40–51; fraction 3, tubes 52–60; fraction 4, tubes 61–71. Fraction 1 coincided with the void volume of the column.

tions 0.7:3.0:1.7:3.3:0.7, and in the second case, 0.6:3.0:2.1:3.2:1.9.

Studies on glycopeptide structures. Glycopeptide 6, containing mannose, *N*-acetylglucosamine and aspartic acid in the molar proportions 7:2:1 (Table 1), was incubated with α -mannosidase, and with a mixture of α -mannosidase and β -*N*-acetylglucosaminidase. After 75 h mannosidase alone liberated 4.7 residues of mannose, whereas when both enzymes were present 6.5 residues were liberated, together with 1.2 residues of *N*-acetylglucosamine. At the end of the incubation period, the control experiments showed that mannosidase retained its activity, although that of *N*-acetylglucosaminidase had been lost. Protein material was separated from the incubation mixture by exclusion chromatography on Sephadex G-50. The

included ninhydrin-positive fractions were pooled, concentrated and fractionated on Sephadex G-15. Peptide 'mapping' of the pooled ninhydrin-positive fractions which contained no free monosaccharides showed that a single glycopeptide was present. This glycopeptide had mol.wt. 850 as estimated by exclusion chromatography and contained mannose, *N*-acetylglucosamine and aspartic acid in the molar proportions 1.7:2.0:1.0.

One mol of glycopeptide 6 rapidly consumed about 6 mol of periodate (Fig. 3), and the oxidized glycopeptide contained mannose, *N*-acetylglucosamine and aspartic acid in the molar proportions 2.3:2.1:1.0.

Glycopeptide 7, containing fucose, mannose, galactose, *N*-acetylglucosamine, sialic acid and aspartic acid in the molar proportions 1:3:2.5:1:1 was incubated with β -*N*-acetylglucosaminidase. No monosaccharide was released, although the enzyme was active against glycopeptides as shown by the liberation of *N*-acetylglucosamine from ovalbumin glycopeptide. Periodate oxidation of the glycopeptide destroyed all the fucose, galactose and sialic acid, and one residue of mannose, but none of the *N*-acetylglucosamine.

DISCUSSION

Don IgM is an example of a type L macroglobulin but its carbohydrate composition did not differ significantly from that of three type K macroglobulins studied by Spragg & Clamp (1969).

To carry out structural studies on the oligosaccharide units of this protein, large amounts of the electrophoretically pure glycopeptides were prepared. The carbohydrate composition of glycopeptides is sometimes calculated relative to the content

Table 3. *Amino acid and carbohydrate composition of S-carboxymethylcysteine-containing glycopeptides*

IgM was reduced and alkylated and then digested with trypsin. The S-carboxymethylcysteine (CMCys) glycopeptides were obtained by high-voltage electrophoresis of Sephadex fraction 3 (Fig. 2). The compositions of glycopeptides $\mu 3$ and $\mu 5$ described by Beale & Butters (1969) are shown for comparison.

Residue	Composition (mol/mol of glycopeptide)			
	Don IgM		$\mu 3$	$\mu 5$
Asp	3.1	2.7	2.9	3.1
Thr	1.8	1.6	1.8	2.2
Ser	2.6	1.9	2.9	2.1
Glu	2.8	2.8	2.8	3.2
Pro	1.1	1.5	0.8	1.0
Gly	2.2	1.2	2.2	2.1
Ala	1.8	1.3	2.0	1.8
Val	1.3	1.1	1.0	0.8
Met	0.8	0.4	0.0	0.0
Ile	1.0	1.1	0.9	0.8
Leu	1.3	1.2	0.0	0.0
Tyr	0.0	0.0	0.0	0.0
Phe	1.1	0.9	1.0	0.8
Lys	0.0	0.0	0.0	0.0
His	0.0	0.0	0.8	0.7
Arg	0.9	0.9	0.9	1.0
CMCys	+	+	+	+
Fucose	0.7	0.6		
Mannose	3.0	3.1		
Galactose	1.7	2.1		
N-Acetylglucosamine	3.3	3.2		
Sialic acid	0.7	1.9		

of aspartic acid, which in glucosamine-containing glycopeptides is usually the site of attachment of the oligosaccharide unit. This may give misleading results when glycopeptides are obtained by the action of a non-specific protease. Thus a particular oligosaccharide unit might give rise to a number of glycopeptides differing in the length of attached polypeptide chain, which may contain an additional aspartic acid residue (Spragg & Clamp, 1969). The molar composition of each glycopeptide was therefore determined by a least-squares procedure (Katz, 1968) which can take into account all the residues in the glycopeptides. The results (Tables 1 and 2) show how this procedure assigns glycopeptide compositions which are independent of the errors introduced by molecular-weight estimations, and which do not rely entirely on the amino acid composition. The results described were calculated on the basis of weighting according to relative abundance (Katz, 1968), although the use of other weighting procedures led in all cases but two to molecular weights which differed from those given by not more than 5%. In two examples the differences were of the order of 12%.

Fig. 3. Reduction of 15 mM- NaIO_4 at 20°C in the dark by glycopeptide.

Two types of glycopeptide are present in Don IgM. Some contain fucose, mannose, galactose, N-acetylglucosamine and sialic acid in the approximate molar proportions 1:3-4:2:3-5:0-2, with molecular weights greater than 2000, and these are of the type which we have designated as CA oligosaccharide units in immunoglobulins (Clamp & Johnson, 1971). When glycopeptides of this type are examined for their carbohydrate content (Table 2), it is found that one fucose residue is always present, and with one exception, the glycopeptides contain almost exactly two residues of galactose. All contain almost exactly three or four residues of mannose, and most contain three, four or five residues of N-acetylglucosamine. All these glycopeptides contain the monosaccharides, in amounts which decrease in the order N-acetylglucosamine $\geq$ mannose $>$ galactose $>$ fucose. Several of the glycopeptides contain two residues of aspartic acid, confirming the findings of Spragg & Clamp (1969). In addition, considerable amounts of serine and glutamic acid are present in several of the glycopeptides, suggesting that these amino acids are close to one or more of the attachment sites of the CA oligosaccharide units.

Other glycopeptides found in this IgM are of the type which have been described as Cb oligosaccharide units (Clamp & Johnson, 1971), and contain from six to eight residues of mannose and from two to three residues of N-acetylglucosamine, although the commonest composition is seven and two residues respectively (Table 1). These glycopeptides have molecular weights less than 2000. In addition to aspartic acid, several of the type Cb glycopeptides contain one residue of proline. Proline was also found in a corresponding glycopeptide from Sha

IgM (Spragg & Clamp, 1969). Glycopeptides 1a and 1b have slightly different compositions, accounting for their different electrophoretic mobilities.

The compositions of Don IgM glycopeptides are similar to those reported by Davie & Osterland (1968), but show less variability in the monosaccharide content.

There are more than three glycopeptides representing the CA unit in Don IgM (Table 2) and more than one representing the CB unit (Table 1). These results suggest that heterogeneity is present at each carbohydrate attachment site on the protein chain. Jouanneau *et al.* (1970) during investigation of IgM found similar types of oligosaccharide unit, which also showed heterogeneity.

Beale & Butters (1969) prepared a number of half-cystine-containing glycopeptides from IgM. Two of these had similar amino acid compositions, and were believed to arise from the same site on the μ chain. Similar glycopeptides have been isolated from a number of pathological macroglobulins, as well as from normal human IgM (Beale & Herbert, 1970), and are involved in the disulphide linkage between IgM monomers (Beale & Herbert, 1970). If the oligosaccharide unit present at this site is of the CA type, the different electrophoretic mobilities of these glycopeptides could be due to differences in their sialic acid content. Don IgM was therefore reduced and alkylated, then digested with trypsin, in order to prepare glycopeptides corresponding to those isolated by Beale & Butters (1969). Two glycopeptides were isolated with virtually identical compositions, except that one contained two sialic acid residues, whereas the other contained only one. This may therefore be the explanation for the separation of the two glycopeptides as suggested by Beale & Butters (1969). From these results, it would also appear that Don IgM, and presumably normal human IgM, has a type CA oligosaccharide unit which is attached at a position not greater than about 14 amino acid residues from the intermonomer disulphide bridge.

Structural studies. Type CB oligosaccharide units have been found in IgA and IgM (Dawson & Clamp, 1968; Spragg & Clamp, 1969), and a structure has been proposed for the unit from IgA (Dawson & Clamp, 1968). However, no structural studies on the similar unit from IgM have been reported. Don IgM was therefore used to prepare large amounts of an appropriate glycopeptide, namely glycopeptide 6 (Table 1), which contains 7 residues of mannose, 2 of *N*-acetylglucosamine and 1 of aspartic acid. α -Mannosidase liberated 5 residues of mannose, although when the glycopeptide was incubated with a mixture of α -mannosidase and β -*N*-acetylglucosaminidase, 7 mannose residues and 1 *N*-acetylglucosamine residue were liberated. The end-product of mannosidase action should therefore contain 2

residues of mannose, 2 of *N*-acetylglucosamine and 1 of aspartic acid, and the isolated stub was indeed found to have this composition. As the oligosaccharide unit is almost certainly attached to aspartic acid through *N*-acetylglucosamine, this stub probably has the structure (Man,Man,GlcNAc)-GlcNAc-Asp. The remaining mannose is liberated by mannosidase only after one residue of *N*-acetylglucosamine has been released by β -*N*-acetylglucosaminidase. The glycopeptide stub is therefore probably unbranched with the following structure:

The five mannose residues removed by mannosidase could be attached to this structure in a linear or in a branched way, both of which would be consistent with the results. However, as the glycopeptide consumed 6 mol of periodate for the oxidation of 5 residues of mannose, the structure is probably unbranched. Since all the *N*-acetylglucosamine in the glycopeptide survives oxidation, this chain of mannose residues must be attached to the *N*-acetylglucosamine residue shown to be terminal after the action of α -mannosidase. The glycopeptide is probably therefore an unbranched chain, with the two mannose residues resistant to periodate oxidation substituted at C-3, and the two *N*-acetylglucosamine residues substituted at C-3 or C-4. The structure proposed for the glycopeptide is therefore:

This structure is similar to that proposed by Dawson & Clamp (1968), although the proposed sequence of residues is somewhat different, probably because the earlier studies were carried out on a type CB glycopeptide contaminated to some extent with a type CA unit. It is also possible that this glycopeptide is a mixture of glycopeptides containing similar numbers of residues but with different structures, and in that sense the structure proposed is only tentative.

The CA oligosaccharide units present in Don IgM appear to be different from those in IgA studied by Dawson & Clamp (1968). Thus the IgM glycopeptides contain up to four residues of mannose and up to five residues of *N*-acetylglucosamine, whereas the IgA units contain up to three and four residues of these monosaccharides respectively. Dawson & Clamp (1968) found that the *N*-acetylglucosamine in excess of 3 mol/mol of glycopeptide was in a terminal position, being susceptible to periodate oxidation and the action of β -*N*-acetylglucosaminidase. All the *N*-acetylglucosamine in glycopeptide 7 survived periodate oxidation, and none was released by β -*N*-acetylglucosaminidase, suggesting that there is no terminal *N*-acetylglucosamine in this glycopeptide. In other respects, the results are

Table 4. *Number of oligosaccharide units in Don IgM monomer*

The mean composition of the oligosaccharide units is calculated from the sum of the products of the molar yields and composition of the glycopeptides given in Tables 1 and 2. The theoretical value for the total residues given in IgM monomer is the sum of the residues in the postulated oligosaccharide units.

	No. of units	No. of monosaccharide residues			
		Ca type	Cb type	Total residues	
				Theoretical	Original protein
		6	2		
Fucose		1	0	6	6
Mannose		3	7	32	35
Galactose		2	0	12	11
N-Acetylglucosamine		4	2	28	27
Sialic acid		1	0	6	9

compatible with those of Dawson & Clamp (1968), which indicated that fucose, galactose and sialic acid are in terminal positions. Two mannose residues survive periodate oxidation in the Ca-type unit. This is similar to the results with the Cb unit, suggesting that the two types of oligosaccharide may have some structural similarities.

The number of oligosaccharide units of the Ca and Cb types in IgM monomer is given in Table 4. The results suggest that there are six oligosaccharide units of the Ca type and two of the Cb type. This protein is therefore very similar to Tya IgM described by Spragg & Clamp (1969).

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