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A Study of the Carbohydrate Present in Three Type K Macroglobulins

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For a monomeric molecular weight of 180000 three type K macroglobulins (IgM) contained 6-deoxygalactose, mannose, galactose, 2-acetamido-2-deoxyglucose and *N*-acetylneuraminic acid in the molar proportions 5:38:11:27:7 for Row IgM, 5:31:9:21:7 for Sha IgM, and 5:29:11:26:8 for Tya IgM. The first two proteins were euglobulins whereas Tya IgM was a pseudoglobulin, and therefore the total content of carbohydrate does not appear to be related to the physicochemical properties of the proteins. The three proteins appeared to contain different numbers of oligosaccharide units, Row IgM having about ten units/monomer, and Sha IgM and Tya IgM about eight each. All three proteins had two types of oligosaccharide unit, which by analogy with an immunoglobulin A myeloma globulin were called Type 2 and Type 3 respectively. The Type 2 units had molecular weights equal to or greater than 2000 and contained 1 residue of 6-deoxygalactose, 3–4 of mannose, 1–2 of galactose, 3–4 of 2-acetamido-2-deoxyglucose and 0–2 of *N*-acetylneuraminic acid. The Type 3 units had molecular weights of less than 2000 and contained 0–1 residue of 6-deoxygalactose, 3–6 of mannose, 0–1 of galactose, 1–3 of 2-acetamido-2-deoxyglucose and no *N*-acetylneuraminic acid. Glycopeptides corresponding to the two types of unit varied in their aspartic acid content in that most of the Type 3 glycopeptides possessed only 1 residue of aspartic acid whereas most of the Type 2 glycopeptides had an average content greater than 1 residue.

Despite the relatively low content of IgM† in normal human serum, amounting to only approx. 130mg./100ml. (Eastham, 1967), this immunoglobulin plays a significant part in the immunological system (Cohen & Porter, 1964). Much information has been obtained on the properties and structure of IgM by studies on the protein isolated from the serum of patients with Waldenström's macroglobulinaemia. The IgM immunoglobulins produced in this condition are similar to myeloma globulins in that they are the product of malignant cells and are therefore less heterogeneous than the normal pooled protein, although the physicochemical properties appear to be similar (Muller-Eberhard & Kunkel, 1959). IgM can be reduced by mercaptans to yield monomeric units with a molecular weight of approx. 180000 (Deutsch & Morton, 1958; Miller & Metzger, 1965*a*), which have the same percentage of carbohydrate as the undissociated molecule (Kunkel, 1960). As with other classes of immunoglobulins so far examined, the IgM monomer appears to contain four polypeptide chains, consisting of two heavy and two light chains (Cohen, 1963; Chaplin, Cohen & Press, 1965). These chains are linked by interchain disulphide bonds (Miller & Metzger, 1965*b*), as in IgG (Porter, 1962), and similar linkages are believed to join the five monomeric units present in the undissociated molecule (Miller & Metzger, 1965*b*; Morris & Inman, 1968). Although IgM contains more carbohydrate than do IgG and IgA, little is known about the types of oligosaccharide units that are present in this protein. IgG is believed (Clamp & Putnam, 1964) to contain an average of one oligosaccharide unit in each heavy chain (γ-chain) whereas IgA heavy chain (α-chain) contains three (Dawson & Clamp, 1968). IgA contains three types of oligosaccharide unit, two of which are linked *N*-glycosidically to aspartic acid whereas the third is linked *O*-glycosidically to serine or threonine. IgM has been reported (Miller & Metzger, 1965*a*) to contain 10·22% of total carbohydrate, although another

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

study (Davie & Osterland, 1968) has reported two types of IgM containing 7·71% and 10·69% respectively. This carbohydrate content partly explains the higher molecular weight of the μ-chain compared with the γ-chain. Bourrillon & Razafimahaleo (1967) have reported that IgM contains five or six oligosaccharide units distributed on various parts of the μ-chain.

This study of three IgM proteins was undertaken to detect any differences between the carbohydrate contents of IgM euglobulins and pseudoglobulins and to determine the number and types of oligosaccharide units present in each protein.

MATERIALS

Solids for standards were dried to constant weight under vacuum over P_2O_5. Carbohydrates were obtained from BDH Chemicals Ltd. (Poole, Dorset), except for *N*-acetylneuraminic acid, which was obtained from Sigma (London) Chemical Co. Ltd. (London S.W.6). Pronase (B grade) was supplied by Calbiochem Ltd. (London W.1). Sephadex and Dextran Blue were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden).

We thank Dr A. E. Read and Dr J. Verrier Jones (Department of Medicine, University of Bristol) for sera from patients with Waldenström's macroglobulinaemia.

METHODS

Preparation of IgM. Serum from patients Row and Sha suffering from Waldenström's macroglobulinaemia was dialysed against water at 4° for 24hr. The resultant precipitate was isolated by centrifugation, dissolved in 0·15M-NaCl and the protein reprecipitated as before. The euglobulin precipitations were repeated four times. Tya IgM, which was a pseudoglobulin, was isolated and partially purified by repeated salt fractionation in which the material precipitated between 1·0M- and 1·4M-$(NH_4)_2SO_4$ was separated by centrifugation. After isolation, the three macroglobulins (2·5g.) were dissolved in 0·15M-NaCl (10ml.) and subjected to exclusion chromatography on a column (94cm.×2·5cm. diam.) of Sephadex G-150. The effluent was monitored at 280nm. and the IgM fractions, after identification by immunoelectrophoresis, were dialysed exhaustively against water and freeze-dried. The purity of the protein preparations was established by immunoelectrophoresis with a variety of antisera (Dawson & Clamp, 1968).

Preparation and isolation of glycopeptide material. IgM protein (2·0g.) was incubated with Pronase (0·05g.) at 37° in 20mM-$CaCl_2$, pH8·0 (50ml.). The pH was maintained at 8·0 throughout the digestion by addition of 20mM-NaOH. After 24hr. a further addition of Pronase (0·05g.) was made and the digestion continued for a further 24hr. The digest was then adjusted to pH7·0 with 20mM-HCl and after centrifugation the supernatant was freeze-dried. The dried material was extracted with water (20ml.) and subjected to exclusion chromatography in water on a column (140cm.×3·2cm. diam.) of Sephadex G-25 (fine grade). Fractions (5ml.) were collected and the hexose content of a sample (0·1ml.) was determined. The carbohydrate-containing fractions were pooled, freeze-dried (yield approx. 0·4g.) and subjected to further incubation for 24hr. with Pronase (20mg.) under the conditions described above. After neutralization and centrifugation the supernatant was freeze-dried, redissolved in water (10ml.) and applied to a column (140cm.×2·2cm. diam.) of Sephadex G-25 (fine grade) and the hexose-containing tubes, determined as above, were then pooled and freeze-dried. Further purification was performed on prewashed Whatman 3MM filter paper (57cm.×46cm.). A solution of the glycopeptide material (approx. 15mg.) in the minimum volume of water was streaked along the base line with marker spots at each side. This was then subjected to descending chromatography in butan-1-ol–acetic acid–water (12:3:5, by vol.) for 48hr. The marker strips were cut off and stained with ninhydrin. The area corresponding to the ninhydrin-positive base-line material was then eluted with water (Clamp & Putnam, 1964) and freeze-dried. An aqueous solution of the dried material was applied to prewashed Whatman 3MM filter paper (57cm.×46cm.) with marker spots at each side and subjected to high-voltage electrophoresis at 2000v and 100mA for 2hr. with pyridine–acetic acid–water (1:10:289, by vol.), pH3·6. The areas corresponding to ninhydrin-positive spots on the marker strips were eluted from the paper with water and stored at −20°.

Carbohydrate analyses by colorimetric techniques. Standard curves were constructed with monosaccharides in the same molar proportions as in the original protein. 6-Deoxygalactose was determined by the method of Dische & Shettles (1948), hexose by the method of Dubois, Gilles, Hamilton, Rebers & Smith (1956), hexosamine by the Boas (1953) modification of the Elson & Morgan (1933) method after hydrolysis of the sample at 100° in 2M-HCl for 6hr. and free *N*-acetylneuraminic acid by the Warren (1959) method after hydrolysis of the sample with 0·05M-H_2SO_4 at 80° for 1hr.

Colorimetric procedures were only used to monitor the carbohydrate content of the column effluent for comparison of monosaccharide peaks in Fig. 1. Under the hydrolytic conditions used the release of aminohexose may not have been quantitative.

Carbohydrate analyses by g.l.c. The carbohydrate content of the proteins and glycopeptides was determined by g.l.c. by the method of Clamp, Dawson & Hough (1967).

Amino acid analyses. The glycopeptides were hydrolysed with 6M-HCl for 22hr. at 110°. The dried hydrolysates were then kindly examined by Dr B. T. Pickering, Department of Pharmacology, University of Bristol, for neutral and acidic amino acids by the method of Spackman, Stein & Moore (1958). Sha glycopeptide 1 was also analysed for basic amino acids by the same method.

Determination of the molecular weights of glycopeptides. The molecular weight of each glycopeptide was determined by the method of Bhatti & Clamp (1968). In this procedure a solution of the glycopeptide (0·1mg.) together with D-glucose (0·1mg.) and Dextran Blue (0·1mg.) in 0·15M-NaCl (0·2ml.) was applied to a column (150cm.×1·2cm. diam.) of Sephadex G-50, which was then eluted with 0·15M-NaCl. The molecular weight of the glycopeptide was estimated from its elution volume relative to those of D-glucose and Dextran Blue, the column having been previously calibrated with glycopeptides of known molecular weight.

RESULTS

The macroglobulins Row IgM, Sha IgM and Tya IgM when isolated appeared pure by starch-gel

Table 1. *Carbohydrate content of IgM*

Experimental details are given in the text. The results are the mean of eight determinations and are expressed as the number of monosaccharide residues/mol. of IgM monomer (mol.wt. 180000).

	Carbohydrate composition		
Antigenic type	Row IgM K	Tya IgM K	Sha IgM K
Physicochemical properties	Euglobulin	Pseudoglobulin	Euglobulin
Monosaccharide			
6-Deoxygalactose	5·4±0·4	5·2±0·5	5·2±0·4
Mannose	38·1±2·9	28·9±2·2	31·2±2·5
Galactose	10·7±0·8	10·7±0·8	9·2±0·7
2-Acetamido-2-deoxyglucose	27·4±2·5	26·3±2·3	20·9±2·5
N-Acetylneuraminic acid	7·4±0·6	7·6±0·7	6·9±0·9

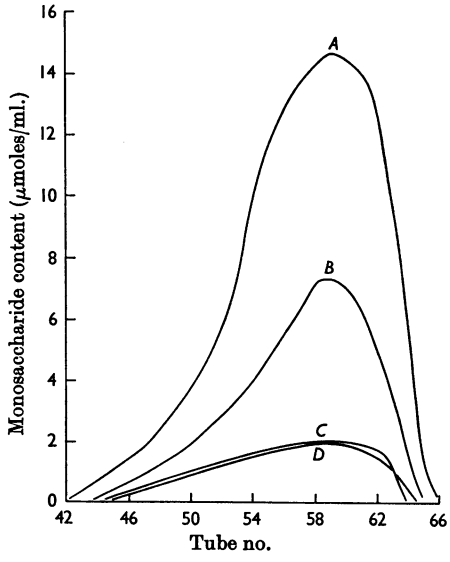


Fig. 1. Chromatography on Sephadex G-25 of a Pronase digest of Row IgM. Samples from each tube were assayed for (*A*) hexose content by the method of Dubois *et al.* (1956); (*B*) aminohexose by the method of Boas (1953); (*C*) sialic acid by the method of Warren (1959); (*D*) fucose by the method of Dische & Shettles (1948). Fractions were 5ml.

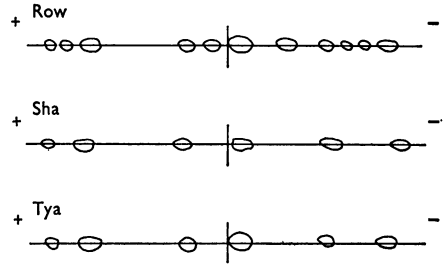


Fig. 2. Separation by high-voltage electrophoresis of the glycopeptides obtained from Pronase digestions of Row IgM, Sha IgM and Tya IgM.

electrophoresis, disc electrophoresis and immunoelectrophoresis with a variety of antisera. All three proteins were type K and showed no reaction with type L antiserum and thus were not significantly contaminated with normal IgM or other immunoglobulins. Row IgM and Sha IgM were euglobulins whereas Tya IgM was a pseudoglobulin. All three proteins contained 6-deoxygalactose, mannose, galactose, 2-acetamido-2-deoxyglucose and *N*-acetylneuraminic acid in the proportions shown in Table 1. The effluent after exclusion chromatography of the second Pronase digest was analysed by colorimetric techniques for 6-deoxygalactose, neutral hexose, hexosamine and *N*-acetylneuraminic acid. The results showed (Fig. 1) almost symmetrical single peaks, that coincided in all four analyses. The glycopeptide material after exclusion chromatography contained a number of contaminating peptides that were removed by descending chromatography on Whatman 3MM filter paper in butan-1-ol–acetic acid–water. The glycopeptides were finally separated according to their electrophoretic mobilities, yielding 11 glycopeptides in Row IgM and six in both Sha IgM and Tya IgM (Fig. 2). The electrophoretically separated glycopeptides were eluted from the paper and stored at −20° until required. Samples of each glycopeptide solution were taken for carbohydrate and amino acid analyses and the results were calculated as monosaccharide residues/mol. of aspartic acid. These results suggested that a number of glycopeptides contained more than 1 residue of aspartic acid. The molecular weights were therefore determined by exclusion chromatography (Table 2). The elution profile of the peaks during this procedure indicated that the glycopeptides were still heterogeneous (Fig. 3). The ratio of the molecular weight

Table 2. *Comparison of molecular and equivalent weights of the glycopeptides*

Experimental details are given in the text. The molecular weight was determined by exclusion chromatography and the equivalent weight calculated from the aspartic acid content, which was assumed to represent 1 residue. The ratio of the molecular weight to the equivalent weight represents the actual aspartic acid content as shown in the final column.

Glycopeptide no.		Mol.wt.	Equiv.wt.	Aspartic acid content (moles/mole of glycopeptide)
Row	1	1550	1431	1·08
	2	1700	1681	1·01
	3	1650	1048	1·57
	4	1450	1007	1·44
	5	1450	1225	1·18
	6	2050	1344	1·53
	7	2150	1477	1·46
	8	2000	1436	1·39
	9	2150	1501	1·43
	10	2050	1069	1·92
	11	2050	1133	1·81
Sha	1	1450	1393	1·04
	2	1700	1724	0·99
	3	1400	1610	0·87
	4	2050	1760	1·17
	5	2150	1352	1·59
	6	2100	1242	1·69
Tya	1	1700	1664	1·02
	2	1400	1357	1·03
	3	2100	1619	1·30
	4	1900	1248	1·52
	5	2350	2359	1·00
	6	2150	1571	1·37

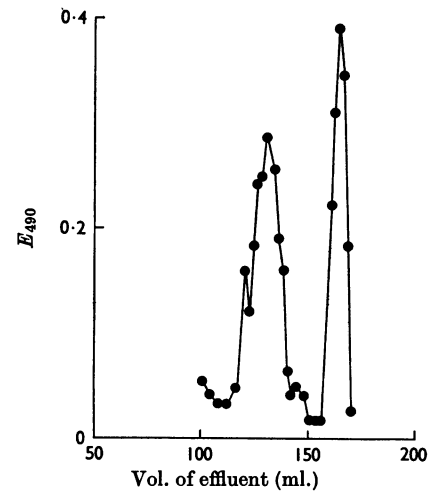


Fig. 3. Chromatography on Sephadex G-50 of Row glycopeptide 4. The glycopeptide was eluted between 120 and 140 ml. of effluent and D-glucose was eluted between 160 and 170 ml. of effluent. Each tube was assayed for hexose content by the method of Dubois *et al.* (1956).

determined by exclusion chromatography to the equivalent weight calculated from the aspartic acid content represents the number of residues of this amino acid in the glycopeptide (Table 2). The number of monosaccharide residues/mol. of glycopeptide and the relative molar yield of each glycopeptide are shown in Table 3.

DISCUSSION

Row IgM and Sha IgM differ markedly from Tya IgM in physicochemical properties in that the first two are euglobulins whereas Tya IgM is a pseudoglobulin. As all three proteins are IgM type K, the *C*-terminal portions of each light and heavy chain are likely to be similar (Cohen & Milstein, 1968). The different properties must therefore arise either from the variable portion of the polypeptide chains or from the carbohydrate content. However, Tya IgM contains an amount of carbohydrate intermediate between those of Row IgM and Sha IgM (Table 1), namely 5 residues of 6-deoxygalactose, 29 of mannose, 11 of galactose, 26 of 2-acetamido-2-deoxyglucose and 8 of *N*-acetylneuraminic acid. The results indicate that the total amount of carbohydrate in these macroglobulins is not directly related to their physicochemical characteristics, although other factors such as position or structure of the oligosaccharide units could still play some part.

The Pronase digests of the proteins were subjected to exclusion chromatography on a column of Sephadex G-25 and the effluent was examined for the content of each of the monosaccharide classes. The profile (Fig. 1) showed that in each case the peak was essentially symmetrical with no obvious separation of glycopeptides of different composition. The purified glycopeptide material from Row IgM separated into 11 components on high-voltage electrophoresis, whereas Sha IgM and Tya IgM both yielded six glycopeptides (Fig. 2). There is no direct relationship between the number of glycopeptides that are found upon electrophoresis and the number of oligosaccharide units in the original protein. The only relationship appears to be an indefinite one in that, as might be expected, the larger the number of units in a glycoprotein, the more glycopeptides will be found electrophoretically, and this general trend is shown in the three macroglobulins. Glycopeptides are not as sensitive as peptides in the ninhydrin staining reaction and the number that are seen depends to some extent on

Table 3. *Composition of glycopeptides from Row IgM, Sha IgM and Tya IgM*

Experimental details are given in the text. The relative yields of glycopeptides are given as moles/100 moles of total glycopeptides for each protein. The compositions of the glycopeptides are given as moles/mole of glycopeptide and the results, which were determined by single analyses for amino acids and carbohydrate, are expressed as: Fuc, 6-deoxygalactose; Man, mannose; Gal, galactose; GlcNAC, 2-acetamido-2-deoxyglucose; NANA, *N*-acetylneuraminic acid. Components present in amounts less than 0·1mole are denoted as trace (tr.). The glycopeptides are numbered according to their increasing mobility towards the anode.

Glycopeptide no.		Yield of glycopeptide (moles/100moles)	Composition (moles/mole of glycopeptide)											
			Fuc	Man	Gal	GlcNAC	NANA	Asp	Ser	Thr	Pro	Val	Glu	Ala
Row	1	11·9	0	5·9	0	1·5	0	1·1	0·7	0·2	0	0·5	tr.	tr.
	2	13·0	0·5	4·1	0·8	2·8	0	1·0	0·3	0·5	tr.	0·2	0·1	tr.
	3	2·5	0·5	3·3	0·6	2·5	0	1·6	1·1	0·2	tr.	0	0·8	tr.
	4	4·0	0·3	4·2	0·4	2·2	0	1·4	0	0·1	0	tr.	tr.	tr.
	5	7·1	0·3	4·5	0·5	1·9	0	1·2	0·2	0·7	tr.	0	0·2	tr.
	6	16·4	1·1	3·5	2·0	3·2	0·2	1·5	0·6	0·3	0	0	0	0·3
	7	21·2	0·9	3·4	1·6	3·2	1·2	1·5	0·4	tr.	0	0	tr.	tr.
	8	4·5	1·0	3·5	1·4	3·3	0·6	1·4	0·3	tr.	0	0	0·1	tr.
	9	16·1	0·7	2·7	1·7	3·2	1·3	1·4	0·3	0·3	0	0	0·4	tr.
	10	2·2	1·0	3·1	1·5	3·1	0·6	1·9	0·2	tr.	0	tr.	0·6	0·4
	11	1·1	0·7	2·5	1·6	2·7	1·5	1·8	0	0·5	0	0	0	tr.
Sha	1	15·2	0·2	5·1	0	1·7	0	1·0	0·4	tr.	0·8	0	tr.	tr.
	2	12·4	0·3	5·0	0·6	2·5	0	1·0	0·4	0·3	0	0·5	0·1	tr.
	3	14·5	0·3	6·1	0	1·2	0	0·9	tr.	tr.	0	0	tr.	tr.
	4	27·6	0·8	2·7	1·5	3·6	0·9	1·2	0·2	0·3	0	0	0·3	tr.
	5	20·0	1·0	3·0	1·9	3·2	0·8	1·6	0·3	0·3	0	0	0·4	tr.
	6	10·3	0·8	3·0	1·8	3·4	0·5	1·7	0·2	tr.	0	0	0·9	tr.
Tya	1	20·2	0·3	5·4	0·6	2·0	0	1·0	0·5	0·3	0	0·5	tr.	tr.
	2	9·7	0·4	4·8	0·6	1·4	0	1·0	0·2	tr.	0	0	tr.	tr.
	3	33·6	0·9	3·1	2·0	3·6	0·5	1·3	0·4	0·2	0	0	0·1	0·1
	4	10·4	0·8	3·0	1·7	3·0	0·6	1·5	0·3	tr.	0	0	0·1	tr.
	5	21·6	0·8	2·8	1·9	3·8	1·6	1·0	0·3	0·3	0	0	0·3	0·2
	6	4·5	1·4	3·3	2·3	3·3	0·4	1·4	0·1	tr.	0	0	0·4	0

the amount of material available for loading the chromatogram. More material was available from Row IgM than from Sha IgM and Tya IgM, and minor glycopeptides representing as little as 1 mole/100 moles of total glycopeptides (Row glycopeptide 11) could therefore be isolated and analysed.

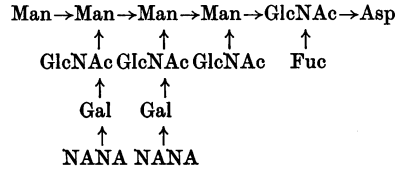
The mobility of any particular glycopeptide in this system is the resultant of many factors. The presence of a single hydrophobic or hydrophilic amino acid residue in a glycopeptide can affect the mobility (Dawson & Clamp, 1968). The effect of negative charge contributed by aspartic acid or *N*-acetylneuraminic acid residues is discussed below. Positive charge could be contributed by basic amino acids or, less likely, by unacetylated glucosamine. Row glycopeptide 1, Sha glycopeptide 1 and Tya glycopeptide 1, which appear (Fig. 2) to have mobilities towards the cathode, are probably carried to those positions by endosmosis. Sha glycopeptide 1 was analysed for basic amino acids and none were found, although the possibility of the presence of unacetylated glucosamine was not investigated.

The equivalent weight of the glycopeptide, calculated from the aspartic acid content, is in many cases identical with the molecular weight (Clamp & Putnam, 1967; Dawson & Clamp, 1968). However, when the carbohydrate analyses of the macroglobulin glycopeptides were related to the aspartic acid content the results were complex and difficult to interpret. Thus for example the numbers of monosaccharide residues in some glycopeptides such as Row glycopeptides 10 and 11 were about half those present in others such as Tya glycopeptide 5. A method based on exclusion chromatography was therefore developed (Bhatti & Clamp, 1968) for the estimation of the molecular weights of glycopeptides that could be carried out on the small amounts of material that were available. The molecular weights obtained by this method are shown in Table 2, together with the equivalent weights calculated from the aspartic acid content. The ratio of the molecular weight to the equivalent weight approximates to the number of aspartic acid residues in the glycopeptide and this varies from 1·0

in Row glycopeptides 1 and 2, Sha glycopeptides 1 and 2 and Tya glycopeptide 5 to almost 2·0 in Row glycopeptides 10 and 11 (Table 2). The monosaccharide contents were therefore calculated according to the chromatographically determined molecular weight as shown in Table 3. As the electrophoretic mobility of the glycopeptides cannot be strictly related to the *N*-acetylneuraminic acid content it is possible that in certain glycopeptides the extra aspartic acid may have a free ω-carboxyl group. Thus there is a mobility difference between Row glycopeptides 8 and 10 although they have similar contents of *N*-acetylneuraminic acid and the same is true of Row glycopeptides 9 and 11. Since Row glycopeptides 9 and 11 differ by approx. one *N*-acetylneuraminic acid residue from Row glycopeptides 8 and 10 it appears that aspartic acid contributes to the electrophoretic mobility in some or all of the four glycopeptides. Variability in the aspartic acid content might arise if similar oligosaccharide units were present on different sites of the heavy chain, some of which had an adjacent aspartic acid residue. Heterogeneity is also reflected in the contents of monosaccharides other than *N*-acetylneuraminic acid which in many glycopeptides do not correspond to an integral number of residues. Direct evidence for heterogeneity was found during exclusion chromatography for molecular-weight determinations, when the glycopeptide peaks were found to be asymmetrical (Fig. 3).

Although Sha IgM and Tya IgM macroglobulins contain less carbohydrate than Row IgM and yield fewer glycopeptides all three proteins contain a similar range of oligosaccharide units. These can be divided into two broad types, according to their molecular weights and carbohydrate contents (Table 3). The first type includes Row glycopeptides 1–5, Sha glycopeptides 1–3 and Tya glycopeptides 1 and 2, which have a molecular weight significantly less than 2000 and contain less than 0·5 residue of 6-deoxygalactose, more than 3 residues of mannose, less than 1 residue of galactose, less than 3 residues of 2-acetamido-2-deoxyglucose and no *N*-acetylneuraminic acid. Of these, Row glycopeptide 1 and Sha glycopeptides 1 and 3 are unique in that they contain 5–6 residues of mannose, 1–2 residues of 2-acetamido-2-deoxyglucose but no galactose. Since Sha glycopeptides 1 and 3 contain in addition significant amounts of 6-deoxygalactose this monosaccharide must be linked to either mannose or 2-acetamido-2-deoxyglucose.

The remaining glycopeptides comprise the second type of oligosaccharide unit with molecular weights of 2000 or more and which contain approx. 1 residue of 6-deoxygalactose, 3–4 of mannose, 1–2 of galactose, 3–4 of 2-acetamido-2-deoxyglucose and 0–2 of *N*-acetylneuraminic acid.



Scheme 1. NANA, *N*-Acetylneuraminic acid; GlcNAc, 2-acetamido-2-deoxyglucose; Fuc, 6-deoxygalactose.

The first type of unit corresponds to some extent with the Type 3 structure described by Dawson & Clamp (1968) containing only mannose and 2-acetamido-2-deoxyglucose, whereas the second type corresponds to the Type 2 oligosaccharide unit, which contains 6-deoxygalactose, mannose, galactose, 2-acetamido-2-deoxyglucose and *N*-acetylneuraminic acid in the proportions 1:3:2:3:0–2. The two types of unit are called Type 3 and Type 2 respectively in the following discussion. In glycopeptides that appear to be Type 2 but have a mannose content greater than 3 residues, such as Row glycopeptides 6, 7 and 8, the extra mannose could be accommodated in the structure proposed by Dawson & Clamp (1968) as shown in Scheme 1.

Since all three proteins contain similar glycopeptides the lower carbohydrate contents of Sha IgM and Tya IgM must be due to the presence of smaller numbers of oligosaccharide units in the original protein. The mean composition (Table 4) of the oligosaccharide units in each protein was calculated from the molar yields and compositions of the individual glycopeptides (Table 3). The agreement between the total carbohydrate calculated from the mean composition of the glycopeptides and that found by analysis in the original protein is remarkably close and indicates that there are about ten oligosaccharide units in Row IgM and eight in each of Sha IgM and Tya IgM (Table 5). An attempt has also been made in Table 5 to give some idea of the numbers of each type of oligosaccharide unit in the original protein.

All the glycopeptides from Sha IgM and Tya IgM with a molecular weight of less than 2000 (Type 3) contained only approx. 1 residue of aspartic acid, whereas those with a molecular weight in excess of 2000 (Type 2), with the exception of Tya glycopeptide 5, contained significantly more than 1 residue of aspartic acid. Resistance to proteolysis might then arise not only because of the steric effect of the carbohydrate (Smyth & Utsumi, 1967) but also because such a sequence may not readily be attacked by Pronase (Nomoto, Narahashi & Murakami, 1960). The Row glycopeptides presented a more complicated picture since a number of the Type 3 glycopeptides contained more than 1

Table 4. *Mean composition of the glycopeptides in Row IgM, Tya IgM and Sha IgM*

The mean glycopeptide composition is calculated from the sum of the products of the molar yields and composition of the glycopeptides from each protein (Table 3). For definition of abbreviations see Table 3. The theoretical content is the mean glycopeptide composition multiplied by the number of oligosaccharide units that gives the closest approximation to the carbohydrate content of the original protein.

	Carbohydrate content (moles/mole of protein or glycopeptide)				
	Fuc	Man	Gal	GlcNAC	NANA
Row IgM					
Mean glycopeptide composition	0·66	3·79	1·23	2·79	0·55
Theoretical content for 10 units	6·6	37·9	12·3	27·9	5·5
Original protein	5	38	11	27	7
Tya IgM					
Mean glycopeptide composition	0·65	3·66	1·51	3·05	0·77
Theoretical content for 8 units	5·2	29·2	12·1	24·4	6·2
Original protein	5	29	11	26	8
Sha IgM					
Mean glycopeptide composition	0·61	3·93	1·05	2·72	0·46
Theoretical content for 8 units	4·9	31·4	8·4	21·8	3·7
Original protein	5	31	9	21	7

Table 5. *Oligosaccharide units of Row IgM, Tya IgM and Sha IgM*

The results are expressed as residues of monosaccharide in each oligosaccharide unit corrected to the nearest whole number. Theoretical value (*a*) is the sum of the residues in the postulated oligosaccharide units, whereas theoretical value (*b*) is the product of the mean glycopeptide composition (Table 4) and the number of oligosaccharide units in each IgM. Both the theoretical values and the values for the original protein are given as monosaccharide residues/IgM monomer (mol.wt. 180000).

	Number of monosaccharide residues					
	Type 3		Type 2	Total residues		
				Theoretical		Original protein
Row IgM				(*a*)	(*b*)	
No. of units	1	3	6			
6-Deoxygalactose	0	0–1	1	7	7	5
Mannose	6	3–4	3–4	38	38	38
Galactose	0	0–1	1–2	12	12	11
2-Acetamido-2-deoxyglucose	1–2	2–3	3–4	28	28	27
N-Acetylneuraminic acid	0	0	0–2	6	6	7
Sha IgM						
No. of units	2	1	5			
6-Deoxygalactose	0–1	0–1	1	6	5	5
Mannose	5–6	5	3	31	31	31
Galactose	0	0–1	1–2	9	8	9
2-Acetamido-2-deoxyglucose	1–2	2–3	3–4	22	21	21
N-Acetylneuraminic acid	0	0	0–1	4	4	7
Tya IgM						
No. of units		2	6			
6-Deoxygalactose		0–1	1	7	5	5
Mannose		5	3	28	29	29
Galactose		0–1	2	13	12	11
2-Acetamido-2-deoxyglucose		1–2	3–4	25	24	26
N-Acetylneuraminic acid		0	0–2	6	6	8

residue of aspartic acid. This may be associated with the fact that Row IgM contained a larger number of oligosaccharide units. Thus the actual numbers of the Type 3 units containing only 1 residue of aspartic acid were very similar in all three IgM proteins, namely two or three for Row IgM, three for Sha IgM and two or three for Tya IgM.

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