Investigations on the Oligosaccharide Units of an A Myeloma Globulin

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The carbohydrate content of an A myeloma globulin was investigated. The carbohydrate content was found to be unchanged when the protein was isolated from the patient over a period of 18 months. The various polymeric forms of the protein contained similar proportions of carbohydrate. The A myeloma globulin contained approx. 2 residues of 6-deoxy-L-galactose (L-fucose), 14–15 of D-mannose, 12–13 of D-galactose, 12–13 of 2-acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine), 6 of 2-acetamido-2-deoxy-D-galactose (*N*-acetyl-D-galactosamine) and 5 of *N*-acetylneuraminic acid (sialic acid), and these were distributed between six oligosaccharide units all of which were present on the heavy polypeptide chains. The oligosaccharide units showed two kinds of heterogeneity, which have been termed central and peripheral. Central heterogeneity was shown by the presence of three completely different core units, which had the following compositions: (1) 3 residues of D-galactose and 3 of 2-acetamido-2-deoxy-D-galactose, joined to protein by an *O*-glycosidic linkage between acetamidohexose and serine; (2) 3 residues of D-mannose, 2 of D-galactose and 3 of 2-acetamido-2-deoxy-D-glucose, joined to protein by an *N*-glycosidic linkage between acetamidohexose and aspartic acid; (3) 4 residues of D-mannose and 3 of 2-acetamido-2-deoxy-D-glucose with a linkage similar to that in (2). The core oligosaccharide units showed peripheral heterogeneity in the attachment of 6-deoxy-L-galactose, 2-acetamido-2-deoxy-D-glucose and *N*-acetylneuraminic acid. Tentative structures are proposed for these various types of oligosaccharide unit. Glycopeptides were isolated in which the sialic acid content exceeded that of D-galactose. Explanations are given for the electrophoretic mobility and staining characteristics of the various glycopeptides.

Although IgA* constitutes only about 10% of the total serum immunoglobulins (Fahey & Lawrence, 1963), it appears to be the major constituent of many secretions such as colostrum (Montreuil, Chosson, Havez & Mullet, 1960), bronchial mucus (Mason & Heremans, 1966), saliva (Tomasi, Tan, Solomon & Prendergast, 1965), tears (Chodirker & Tomasi, 1963) and nasal secretions (Remington, Vosti, Lietze & Zimmerman, 1964). As with other classes of immunoglobulins so far examined, the IgA monomeric unit appears to contain four polypeptide chains, consisting of two heavy and two light chains (Deutsch, 1963; Fahey, 1963), although the possibility of a more complicated structure has been suggested (Franklin, 1964; Bernier, Tominaga, Easley & Putnam, 1965; Suzuki & Deutsch, 1967). These chains are linked (Deutsch, 1963) by a number of interchain disulphide bonds as in IgG (Porter, 1962), and similar linkages may account for the polymerization of the monomeric unit in

IgA (Heremans, Heremans & Schultze, 1959; Deutsch, 1963) and IgM (Miller & Metzger, 1965).

The three major classes of immunoglobulins, IgG, IgA and IgM, contain differing amounts of total carbohydrate (Heremans, 1959); in IgA the amount is approx. 8% (Clamp & Putnam, 1967). Pooled IgG appears to contain an average of two oligosaccharide units/mol. of protein (Clamp & Putnam, 1964), which are believed to be located in the C-terminal half (Fc fragment) of the heavy chain (Franklin, 1960). The linkage between carbohydrate and protein has been postulated (Rosevear & Smith, 1961) to involve 2-acetamido-2-deoxy-D-glucose linked as a glycosylamide to the 4-carboxyl group of aspartic acid. A similar type of linkage has been reported in a number of other glycoproteins, including ovalbumin (Marshall & Neuberger, 1964), orosomucoid (Yamashina, Makino, Ban-I & Kojima, 1965) and ovomucoid (Monsigny & Montreuil, 1966). Glycoproteins may also contain *O*-glycosidic linkages between 2-acetamido-2-deoxy-D-galactose and hydroxy amino acids, as in submaxillary gland

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

glycoproteins (Blix, 1963; Hashimoto, Tsuiki, Nisizawa & Pigman, 1963). IgA has been reported (Heremans, 1959; Clamp & Putnam, 1967) to contain the same types of monosaccharide as IgG, namely 6-deoxy-L-galactose, D-mannose, D-galactose, 2-acetamido-2-deoxy-D-glucose and sialic acid, the relative proportions of which may vary in different myeloma globulins (Muller-Eberhard & Kunkel, 1956; Laurell, Laurell & Waldenström, 1957; Clamp & Putnam, 1967). Preliminary studies (Clamp & Putnam, 1965, 1967) on IgA indicated that the oligosaccharide units were more complicated than in IgG and that there were more than two units in the protein molecule. This study on an IgA myeloma globulin was undertaken to determine the number and structure of the oligosaccharide units and their site on, and mode of linkage to, the protein.

MATERIALS

N-Acetylneuraminidase (500 units/ml.) was obtained from Behringwerke A.-G. (Marburg-Lahn, Germany), β -galactosidase (grade III), pepsin (twice crystallized) and trypsin (type 1, twice crystallized) were from Sigma (London) Chemical Co. Ltd. (London, S.W. 6), β -glucosidase (emulsin) was from Seravac Laboratories Ltd. (Maidenhead, Berks.), galactose oxidase and peroxidase (R.ZO-6) were from Hughes & Hughes (Enzymes) Ltd. (London, W. 1), and Pronase (B grade) and dithiothreitol were from Calbiochem Ltd. (London, W. 1). β -N-Acetylglucosaminidase (3440 000 units/ml.) was kindly supplied by Dr G. A. Levvy of the Rowett Research Institute (Aberdeen) and sialolactase by Professor R. Kuhn of the Max-Planck-Institut (Heidelberg, Germany).

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METHODS

Preparation of the IgA. Blood was taken from a patient with multiple myeloma on two separate occasions at an interval of 18 months (samples A and B respectively). The isolated serum, which contained approx. 5.5 g. of myeloma IgA/100 ml., was dialysed against water at 4° for 24 hr. and then centrifuged. The supernatant was subjected to three salt fractionations in which the material precipitating between 1.0M- and 1.2M-(NH₄)₂SO₄ was isolated by centrifugation. This material (5g.) was dissolved in and dialysed against 20mM-tris-HCl buffer, pH 7.4, and then applied to a column (41 cm. $\times$ 4.3 cm. diam.) of DEAE-cellulose (Whatman DE-22). After the passage of 1500 ml. of the same buffer, a linear gradient was commenced to the

limiting buffer, 0.5M-NaCl-20mM-tris-HCl buffer, pH 7.4. The effluent was monitored at 280 m μ and the IgA-containing fractions, after identification by immunoelectrophoresis, were pooled, dialysed against water and freeze-dried; the yield was approx. 3g. Concentrated solutions (10%, w/v) of the IgA fractions were checked for purity by starch-gel electrophoresis and by immunoelectrophoresis with general antiserum and specific antisera against IgG, IgA and IgM and type K and L light chains. The purified IgA (1g.) was dissolved in 0.15M-NaCl (40 ml.) and subjected to exclusion chromatography on a column (178 cm. $\times$ 4.3 cm. diam.) of Sephadex G-150. The peaks containing IgA were dialysed against water and freeze-dried.

Separation of heavy and light polypeptide chains. The IgA (0.5g.) was reduced with dithiothreitol (0.25g.) in 0.5M-tris-acetate buffer, pH 8.0 (50 ml.) for 1 hr. (Cleland, 1964) and alkylated with a molar excess of iodoacetic acid. The reaction mixture was then subjected to exclusion chromatography on a column (50 cm. $\times$ 2.0 cm. diam.) of Sephadex G-25 equilibrated with 0.1M-phosphate buffer, pH 6.5. The protein-containing effluent was concentrated by pressure-dialysis against 1.0N-acetic acid, and the heavy and light chains were separated by exclusion chromatography on a column (138 cm. $\times$ 3.5 cm. diam.) of Sephadex G-100 in 1.0N-acetic acid.

Preparation and isolation of glycopeptide material. The IgA (5.0g.) was incubated with Pronase (0.15g.) in 20mM-CaCl₂, pH 8.0 (75 ml.). The pH was maintained at 8.0 with 20mM-NaOH, and after 24 hr. a second addition of Pronase (0.15g.) was made and the digest was continued for a further 24 hr. After centrifugation, the supernatant was freeze-dried and the dried material (4.8g.) extracted with water (20 ml.). This solution was subjected to exclusion chromatography on a column (140 cm. $\times$ 3.2 cm. diam.) of Sephadex G-25 (fine grade) (Pharmacia Fine Chemicals, Uppsala, Sweden). Fractions (5 ml.) were collected and the hexose content of a sample (0.1 ml.) from each tube was determined. The carbohydrate-containing fractions were pooled, freeze-dried (yield 0.9g.) and subjected to further incubation with Pronase (0.04g.) for 24 hr. as described above. The digest was concentrated by freeze-drying, and exclusion chromatography was then performed on a column (140 cm. $\times$ 2.2 cm. diam.) of Sephadex G-25 (fine grade) and the effluent monitored for hexose as before. A final purification of the glycopeptide material was achieved on pre-washed Whatman 3MM filter paper (57 cm. $\times$ 46 cm.; 25 mg. of glycopeptide/chromatogram) by descending chromatography for 48 hr. in butan-1-ol-acetic acid-water (12:3:5, by vol.), followed, after drying, by elution of the base-line material with water (Clamp & Putnam, 1964). The glycopeptides were then isolated according to their electrophoretic mobilities: in this procedure, the glycopeptide material was applied to pre-washed Whatman 3MM filter paper (57 cm. $\times$ 46 cm.; 25 mg. of glycopeptide/electrophoretogram) and subjected to electrophoresis for 3 hr. at 2000 V and 100 mA in pyridine-acetic acid-water (1:10:289, by vol.), pH 3.6. The areas corresponding to ninhydrin-positive spots were eluted from the paper with water, pooled with corresponding material from duplicated or triplicated electrophoretic runs, and freeze-dried. The purity of the isolated glycopeptide was checked by electrophoresis.

The IgA (1.8g.) was digested with pepsin (20 mg.) for 60 hr. in water (50 ml.) and adjusted to and maintained at

pH 2.0 with 2N-HCl, and further additions of pepsin (20 mg.) were made after 6 and 24 hr. The pH of the digest was adjusted to 7.6 with 2N-NaOH, and then trypsin (20 mg.) was added and the solution was incubated for 48 hr. The digest was freeze-dried and extracted with water (10 ml.), and glycopeptide material was isolated as described above.

Chromatograms were stained with freshly prepared 0.1% ninhydrin in acetone, and the colour was developed at 80° for 10 min. Glycopeptides were identified by the periodate-ammoniacal AgNO₃ technique (Clamp & Putnam, 1964).

Carbohydrate analyses by colorimetric techniques. Calibration curves for monosaccharide determinations were constructed from mixtures of 6-deoxy-L-galactose, D-mannose, D-galactose, 2-amino-2-deoxy-D-glucose and N-acetylneuraminic acid in the proportions present in the original protein. Hexose was determined by the method of Dubois, Gilles, Hamilton, Rebers & Smith (1956) and 6-deoxy-L-galactose by the method of Dische & Shettles (1948). Acetamidohexose was determined by the Morgan & Elson (1934) method and aminohexose by the Boas (1953) modification of the Elson & Morgan (1933) procedure after hydrolysis of the sample in 2N-HCl at 98–100° for 6 hr. N-Acetylneuraminic acid, released by neuraminidase or mild acid hydrolysis (0.1N-H₂SO₄ at 80° for 1 hr.), was estimated by the Warren (1959) procedure.

Carbohydrate analyses by gas-liquid chromatography. 6-Deoxy-L-galactose, D-mannose, D-galactose, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose and N-acetylneuraminic acid were determined as the trimethylsilyl ethers of the methyl glycosides by the method of Clamp, Dawson & Hough (1967). In this procedure, the material to be analysed (approx. 1 mg.), together with the internal standard D-mannitol (0.05 mg.), in a 10 ml. pear-shaped flask, was heated under reflux on an oil bath with N-HCl in dry methanol at 80° for 24 hr. The solution was then neutralized with Ag₂CO₃, and N-acetylation was performed in the same flask by reaction with acetic anhydride (0.1 ml.) at room temperature for 24 hr. After evaporation of the filtrate under reduced pressure at 40°, the dried material was triturated with pyridine-trimethylsilylchloride-hexamethyldisilazane (5:1:1, by vol.) (0.05 ml.) for 30 min. A sample (1 μ l.) of the supernatant was injected on to a column (6 ft. $\times$ $\frac{1}{8}$ in. internal diam.) of 3-8% SE30 on Diatoport-S (80–100 mesh). The gas chromatograph (F & M model 400) was temperature-programmed from 140° to 200° at 0.5°/min.

Gas-liquid chromatographic determination of monosaccharides liberated by glycosidases or dilute acid hydrolysis. D-Mannitol (0.20 mg./ml.) as internal standard was added to the glycosidase solutions and to the appropriate monosaccharide standards (0.05–0.20 mg./ml.). Both solutions were treated in the same manner and samples (0.25 ml.) were removed from them at intervals, transferred to a pear-shaped flask (10 ml.) and dried in a vacuum desiccator over P₂O₅. The dried material was silylated and determined as described by Clamp *et al.* (1967).

Determination of terminal non-reducing monosaccharide residues. D-Galactose oxidase or β -galactosidase was used to detect terminal D-galactose residues. Glycopeptides, containing approx. 0.1 mg. of D-galactose, were dissolved in 1.0M-tris-HCl buffer, pH 7.0 (2.0 ml.), and added to a solution of D-galactose oxidase (0.4 mg.), horseradish peroxidase (0.2 mg.) and 1% (w/v) 3,3'-dimethoxybenzidine

in acetone (0.01 ml.) in the same buffer (1.0 ml.). The extinction was followed at 420 m μ , with raffinose (0.005–0.05 mg./ml.) as standard.

Glycopeptides (1 mg.) were incubated with enzymes under the following conditions: β -galactosidase (0.1 mg.), pH 7.2; neuraminidase (50 units) in 0.05M-sodium acetate buffer, pH 5.5; emulsin (2 mg.) in 0.1M-sodium acetate buffer, pH 5.0; β -N-acetylglucosaminidase (34 400 units) in 50 mM-sodium citrate buffer, pH 5.0, containing 0.1M-NaCl and bovine serum albumin (0.02%). The liberated monosaccharides were estimated by gas-liquid chromatography. Glycosidase activity was established by using the following substrates: lactose or raffinose for β -galactosidase, ovalbumin glycopeptide for emulsin and β -N-acetylglucosaminidase and sialolactose for neuraminidase.

Periodate-oxidation studies. Glycopeptide material (1.0 mg.) was dissolved in 15 mM-NaIO₄ (1.0 ml.) and kept in the dark at 20°. Samples (0.05 ml.) were removed at intervals and diluted to 10 ml., and the extinction was measured at 223 m μ (Dixon & Lipkin, 1954). The standard curve was constructed by plotting the E_{223} of a solution of NaIO₃ and NaIO₄ against the molarity of NaIO₄ (J. R. Clamp, G. Dawson & R. B. Nichols, unpublished work). When the consumption of periodate had levelled off, ethylene glycol (1.0 μ l.) and NaBH₄ (1.0 mg.) were added, followed after 24 hr. by D-mannitol (0.05 mg.). The solution was dried in a vacuum desiccator over P₂O₅, and the surviving monosaccharide residues were determined by gas-liquid chromatography.

Detection of O-glycosidic linkages. Glycopeptide material (2.0 mg.) was treated with 0.3M-NaBH₄ in 0.1N-NaOH (2.5 ml.) at 4° for 270 hr. After neutralization with 0.1N-acetic acid, D-mannitol (0.1 mg.) was added and the volume adjusted to 5 ml. Samples (2 ml.) were freeze-dried and subsequently analysed for monosaccharides and amino acids.

Dilute acid hydrolysis. Glycopeptide material was hydrolysed with 0.6N-H₂SO₄ at 85° and the liberated monosaccharides were determined by gas-liquid chromatography. Glycopeptides were also hydrolysed with 20MN-H₂SO₄ at 80°, and samples were taken at intervals up to 2 hr. These were neutralized with solid Ba₂CO₃ and examined by high-voltage electrophoresis.

Molecular-weight determinations. Sedimentation-velocity and equilibrium measurements were made on 0.5% solutions of glycopeptide material in 15 mM-NaCl by using a synthetic-boundary cell in a Spinco model E ultracentrifuge at 59780 and 31800 rev./min. respectively. Molecular weights were calculated by taking 0.70 as the partial specific volume. The molecular weight of glycopeptide material (25 mg.) in water (0.3 ml.) was also calculated from freezing-point depression in an Advanced Osmometer (Fison's Scientific Apparatus Ltd., Loughborough, Leics.).

RESULTS

The IgA (Bra) isolated from blood samples A and B appeared pure by starch-gel electrophoresis and immunoelectrophoresis, but exhibited polymer-type heterogeneity on ultracentrifugation, containing 7s, 11s, 13s and 15s forms in the proportions 35:10:5:1. Preparations A and B were antigenic type K and did not react with type L antiserum. Both preparations contained 6-deoxy-L-galactose,

D-mannose, D-galactose, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose and N-acetylneuraminic acid in the proportions 2:15:13:12:6:5 (Table 1) for a molecular weight of 17 000. Exclusion chromatography of preparation

A on Sephadex G-150 gave three fractions with similar contents of carbohydrate (Table 2). Analyses of the isolated heavy and light polypeptide chains showed that all the carbohydrate was associated with the heavy chain.

Glycopeptide material, after the second Pronase digestion, was separated into three fractions by exclusion chromatography on Sephadex G-25 (Fig. 1). The yields of these three fractions and their contents of carbohydrate are given in Table 3. Sephadex fraction 1 appeared to contain a single glycopeptide when examined by high-voltage electrophoresis (Fig. 2) and when subjected to further exclusion chromatography on Sephadex G-15. Ultracentrifugation also showed the material to be homogeneous. Sephadex fractions 2 and 3 exhibited a considerable degree of heterogeneity on high-voltage electrophoresis (Fig. 2). Thus fraction 2 contained 12 glycopeptides, numbered 1 to 12 in order of increasing anodic mobility, and fraction 3 contained the same glycopeptides with the exception of glycopeptide 4. The relative yield of each glycopeptide is given in Table 4. The glycopeptides appeared to be divisible into three distinct types on the basis of carbohydrate and amino acid analyses.

The first type (glycopeptides 1 and 4) was shown

Table 1. Carbohydrate analyses of IgA

Experimental details are given in the text. The results are expressed as g./100g. of material. Carbohydrate analyses were carried out by gas-liquid chromatography. Colorimetric determinations, where performed, are given in parentheses.

Monosaccharide	Carbohydrate composition (g./100g.)	
	Preparation A	Preparation B
6-Deoxy-L-galactose	0.20 (0.21)	0.19
D-Mannose	1.67	1.68
D-Galactose	1.23	1.36
2-Acetamido-2-deoxy-D-glucose	1.63	1.58
2-Acetamido-2-deoxy-D-galactose	0.83	0.81
N-Acetylneuraminic acid	0.93 (1.01)	0.89

Table 2. Carbohydrate contents of IgA preparations after exclusion chromatography on Sephadex G-150

Experimental details are given in the text. The results are corrected to the nearest residue and are expressed as the number of monosaccharide residues/mol. of IgA protein (mol. wt. 170 000).

Monosaccharide	Carbohydrate content (residues/mol.)				
	Original material	Sephadex G-150 fraction	...	...	
6-Deoxy-L-galactose	2			1	2
D-Mannose	15			14	13
D-Galactose	13			12	12
2-Acetamido-2-deoxy-D-glucose	12			12	12
2-Acetamido-2-deoxy-D-galactose	6			6	6
N-Acetylneuraminic acid	5			4	4

Table 3. Carbohydrate analyses of glycopeptide fractions

Experimental details are given in the text. Glycopeptide material was isolated by exclusion chromatography on Sephadex G-25 after digestion of the IgA with Pronase. Results of the analyses are expressed as g./100g. of material.

Monosaccharide	Sephadex G-25 fraction ...	Carbohydrate content (g./100g.)		
		1	2	3
	Yield (mg.)	149	150	177
6-Deoxy-L-galactose		0	1.6	2.0
D-Mannose		0	12.0	17.6
D-Galactose		15.5	13.6	8.9
2-Acetamido-2-deoxy-D-glucose		0	16.5	23.3
2-Acetamido-2-deoxy-D-galactose		17.9	5.7	2.3
N-Acetylneuraminic acid		0	9.5	13.0

Fig. 1. Chromatography on Sephadex G-25 of a Pronase digest of IgA. Samples from each tube were monitored for hexose content by the method of Dubois *et al.* (1956).

Fig. 2. High-voltage electrophoretic separation of the glycopeptides present in Sephadex G-25 fractions 1, 2 and 3 obtained after Pronase digestion of IgA.

by paper and gas-liquid chromatography to contain D-galactose and 2-acetamido-2-deoxy-D-galactose with the addition of 1 residue of *N*-acetylneuraminic acid in glycopeptide 4, accounting for its greater anodic mobility. Sephadex fraction 1 contained only glycopeptide 1 and this material was used for most of the studies on this type of glycopeptide. Treatment of glycopeptide 1 with dilute alkali in the presence of sodium borohydride caused a significant increase in the content of alanine from 0.5% to 1.6%, whereas α -aminobutyric acid, which would result from an *O*-glycosidic linkage to threonine, was not detected. The D-galactose content was unchanged after this procedure, but about one-third of the 2-acetamido-2-deoxy-D-galactose was destroyed, with the appearance of 2-acetamido-2-deoxy-D-galactitol. The molecular weight of this glycopeptide was 2750 by sedimentation-equilibrium methods and 2350 by

Table 4. Yield of individual glycopeptides from Sephadex G-25 fractions 1, 2 and 3

Experimental details are given in the text. Sephadex G-25 fractions on pre-washed Whatman 3MM filter paper were subjected to electrophoresis for 3 hr. at 2000 v and 100 mA in pyridine-acetic acid-water (1:10:289, by vol.), pH 3.6. The areas corresponding to ninhydrin-positive regions were eluted with water pooled with corresponding material and weighed after freeze-drying. Results are expressed as g./100g. of the total amount isolated.

Glycopeptide no.	Sephadex G-25 fraction ...	Yield of glycopeptide (g./100g.)		
		1	2	3
1		100	19.0	2.0
2			8.0	10.5
3			8.0	17.0
4			12.0	0
5			4.0	6.0
6			1.0	4.0
7			27.0	20.0
8			2.5	19.0
9			1.0	7.0
10			18.0	14.0
11			0.5	1.0
12			0.5	1.0

freezing-point osmometry, suggesting that the glycopeptide contained D-galactose, 2-acetamido-2-deoxy-D-galactose, serine, threonine and proline in the proportions 3:3:4:4:5. The glycopeptide rapidly consumed 5 moles of periodate/mole, which increased to 7 moles/mole after 50 hr. Analysis of the oxidized reduced glycopeptide showed that all the D-galactose residues and 1 of the 3 2-acetamido-2-deoxy-D-galactose residues had been destroyed (Table 5). Incubation of glycopeptide 1 with D-galactose oxidase gave a positive result, which might have been due to terminal D-galactose or 2-acetamido-2-deoxy-D-galactose, since methyl 2-acetamido-2-deoxy- α -D-galactopyranoside was also oxidized by this enzyme. However, the presence of a terminal D-galactose residue was confirmed by the liberation of about one-third of the total D-galactose by β -galactosidase, whereas the β -*N*-acetylglucosaminidase preparation, which also shows β -*N*-acetylglucosaminidase activity (Woollen, Haworth & Walker, 1961), did not liberate any 2-acetamido-2-deoxy-D-galactose. Glycopeptide 4 rapidly consumed 4 moles of periodate/3 moles of D-galactose, and analysis of the oxidized reduced glycopeptide showed that all the sialic acid, 2 out of 3 D-galactose residues and 1 out of 3 2-acetamido-2-deoxy-D-galactose residues had been destroyed (Table 5).

The second type of glycopeptide included glycopeptides 2, 5, 6, 7, 8, 9, 10, 11 and 12, which appeared

Table 5. *Analyses of glycopeptides 1 and 4 before and after oxidation with periodate*

Experimental details are given in the text. The glycopeptides were oxidized with 15 mM-NaIO₄ at 20° in the dark and subsequently reduced with NaBH₄. The results before (a) and after (b) oxidation with periodate are expressed as g./100g. of material.

Monosaccharide	Carbohydrate content (g./100g.)			
	Glycopeptide 1		Glycopeptide 4	
	a	b	a	b
D-Galactose	18.2	0	17.2	4.9
2-Acetamido-2-deoxy-D-galactose	19.4	12.3	20.9	13.8
N-Acetylneuraminic acid	0	0	8.9	0

Table 6. *Composition of glycopeptides 2, 5, 6, 7, 8, 9, 10, 11 and 12*

Experimental details are given in the text. The glycopeptides are numbered according to their increasing anodic mobility. The results are given relative to an aspartic acid content of 1.0 residue. The amino acid content of glycopeptide 11 was not determined (n.d.) after aspartic acid. Colour developed with ninhydrin: br, brown; pu, purple.

Glycopeptide no. ... Component	Composition (moles/mole of aspartic acid)									
	2	5	6	7	8	9	10	11	12	
6-Deoxy-L-galactose	0.3	0.7	0.3	0.1	0.8	0.4	0.6	0.7	0.8	
D-Mannose	3.0	3.0	3.1	3.0	3.1	2.7	3.0	3.0	3.1	
D-Galactose	2.0	2.1	1.9	2.0	2.0	2.0	2.0	2.0	2.0	
2-Acetamido-2-deoxy-D-glucose	3.0	3.0	3.5	3.5	3.5	2.9	3.5	3.0	3.3	
N-Acetylneuraminic acid	0	1.0	1.0	1.0	2.0	2.0	2.0	3.0	3.0	
Aspartic acid	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
Serine	1.5	0.7	0.3	—	—	0.4	—	n.d.	—	
Threonine	1.6	0.7	0.3	—	—	0.2	—	n.d.	—	
Proline	2.8	1.2	0.1	—	—	0.3	—	n.d.	—	
Valine	—	0.7	0.1	—	0.9	0.4	—	n.d.	—	
Glutamic acid	—	—	—	—	—	0.3	—	n.d.	—	
Alanine	—	—	—	—	—	0.1	—	n.d.	—	
Glycine	—	—	0.1	—	—	0.3	—	n.d.	—	
Ninhydrin colour	pu	pu	pu	br	pu	pu	br	pu	br	

to differ from each other in their content of 6-deoxy-L-galactose, N-acetylneuraminic acid and amino acids (Table 6). The glycopeptides with corresponding electrophoretic mobilities from Sephadex fractions 2 and 3 appeared to have identical carbohydrate compositions. The gradual release of N-acetylneuraminic acid by mild acid hydrolysis of glycopeptides 7, 8 and 10 showed only a single intermediate with glycopeptides 8 and 10, whereas glycopeptide 7 did not show an intermediate form, agreeing with the estimated sialic acid content (Table 6). Neuraminidase released between 90 and 100% of the total sialic acid from glycopeptides 7, 8 and 10, and gas-liquid chromatography identified the liberated sialic acid as N-acetylneuraminic acid. The removal of sialic acid from glycopeptides 7, 8 and 10 exposed D-galactose residues, which were then susceptible to β -galactosidase. All the

D-galactose in the neutral glycopeptide 2 was oxidized by D-galactose oxidase. Glycopeptides 7, 8 and 10 rapidly consumed 9, 12 and 12 moles of periodate/mole of aspartic acid respectively. Analysis of the glycopeptides after oxidation showed that all the 6-deoxy-L-galactose, D-galactose and N-acetylneuraminic acid had been destroyed but that half the D-mannose and most of the 2-acetamido-2-deoxy-D-glucose survived (Table 7).

When Sephadex fraction 3 was incubated with β -N-acetylglucosaminidase, 15% of the total content of 2-acetamido-2-deoxy-D-glucose was liberated. After the removal of the acetamido-hexose, the amount of D-mannose oxidized by periodate increased (Table 8).

The third type of glycopeptide, of which glycopeptide 3 was the only example, contained 6-deoxy-L-galactose, D-mannose, D-galactose, 2-acetamido-

Table 7. *Carbohydrate composition of glycopeptides 2, 5, 6, 7, 8, 9 and 10 after oxidation with periodate*

Experimental details are given in the text. The results are expressed as moles/mole of aspartic acid. Analyses were performed on glycopeptides oxidized with 15 mM-NaIO₄ and reduced with NaBH₄.

Glycopeptide no. ...	Composition (moles/mole of aspartic acid)						
	2	5	6	7	8	9	10
Monosaccharide							
6-Deoxy-L-galactose	0	0	0	0	0	0	0
D-Mannose	1.7	1.5	1.4	1.5	1.3	1.4	1.4
D-Galactose	0	0	0	0	0	0	0
2-Acetamido-2-deoxy-D-glucose	2.8	3.0	3.0	2.9	2.8	3.0	3.0
N-Acetylneuraminic acid	0	0	0	0	0	0	0

Table 8. *Carbohydrate analyses of Sephadex G-25 fraction 3*

Experimental details are given in the text. Results are expressed as g./100g. of material and represent the amount before (a) and after (b) oxidation with 15 mM-NaIO₄ of Sephadex G-25 fraction 3, before and after incubation with β -N-acetylglucosaminidase.

Monosaccharide	Carbohydrate content (g./100g. of material)			
	Original material		After incubation	
	a	b	a	b
6-Deoxy-L-galactose	2.0	0	1.9	0
D-Mannose	17.6	10.2	17.8	7.7
D-Galactose	8.9	0	9.1	0
2-Acetamido-2-deoxy-D-glucose	23.3	19.7	19.2	20.2
2-Acetamido-2-deoxy-D-galactose	2.3	0.8	2.5	1.5
N-Acetylneuraminic acid	13.0	0	13.5	0

Table 9. *Carbohydrate and amino acid analyses of glycopeptide 3*

Experimental details are given in the text. The analyses were performed on glycopeptide 3 obtained from Sephadex G-25 fraction 2 (a) and from Sephadex G-25 fraction 3 (b).

Component	Composition (g./100g.) of glycopeptide)	
	a	b
6-Deoxy-L-galactose	0.6	0.5
D-Mannose	29.3	29.2
D-Galactose	4.7	4.1
2-Acetamido-2-deoxy-D-glucose	29.6	28.8
N-Acetylneuraminic acid	0	0
Aspartic acid	5.6	5.7

2-deoxy-D-glucose and aspartic acid in the proportions 0.1:3.9:0.6:3.2:1.0 (Table 9). The glycopeptide rapidly consumed 5 moles of periodate/mole of aspartic acid and had then lost all the D-galactose and between 1 and 2 residues of D-mannose, but none of the 2-acetamido-2-deoxy-D-glucose. Incubation of glycopeptide 3 with β -N-acetylglucos-

aminidase did not appear to release any acetamido-hexose, but incubation with crude emulsin rapidly liberated over 90% of the total D-mannose with a slower release of about 40% of the total 2-acetamido-2-deoxy-D-glucose content.

Glycopeptides were isolated by exclusion chromatography and high-voltage electrophoresis after digestion of the IgA immunoglobulin with pepsin and trypsin. These glycopeptides were partially resolved into fractions A, B and C, which gave results similar (Table 10) to those obtained after digestion with Pronase. Sephadex fraction B was separated into eight discrete glycopeptides on electrophoresis, the cathodic mobility being attributed to basic amino acids in the N-terminal position, and the analyses of these glycopeptides are given in Table 11.

DISCUSSION

Since myeloma globulins are probably produced by a single clone or a small number of clones of malignant plasma cells, they are less heterogeneous than normal immunoglobulins and are therefore more suitable for glycoprotein studies. However,

Table 10. Carbohydrate composition of glycopeptide fractions isolated by exclusion chromatography after pepsin and trypsin digestion of the IgA

Experimental details are given in the text. The IgA (1.77 g.) was incubated with pepsin and trypsin and the digest fractionated by exclusion chromatography on a column of Sephadex G-25.

Sephadex fraction ... Wt. of material (g.) ... Monosaccharide	Carbohydrate content (g./100 g. of material)		
	A	B	C
	0.062	0.088	0.122
6-Deoxy-L-galactose	0.5	0.8	1.0
D-Mannose	3.4	7.7	9.7
D-Galactose	11.6	9.4	5.2
2-Acetamido-2-deoxy-D-glucose	5.0	11.2	11.8
2-Acetamido-2-deoxy-D-galactose	10.5	5.1	1.2
N-Acetylneuraminic acid	1.0	2.9	8.7

Table 11. Carbohydrate analyses of glycopeptides prepared from the IgA after digestion with pepsin and trypsin

Experimental details are given in the text. Glycopeptides were isolated by exclusion chromatography and preparative electrophoresis. The results are given as residues of monosaccharide relative to a D-galactose content of 2.0 residues, and the glycopeptides are numbered according to their increasing anodic mobility.

Glycopeptide no. ... Monosaccharide	Carbohydrate content (moles/2 moles of D-galactose)							
	1	2	3	4	5	6	7	8
6-Deoxy-L-galactose	0	0	0.5	0	0.3	1.3	0	0
D-Mannose	0	0.7	1.6	5.0	3.4	2.4	3.1	3.5
D-Galactose	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
2-Acetamido-2-deoxy-D-glucose	0	0.8	2.3	4.6	3.8	2.7	2.8	3.3
2-Acetamido-2-deoxy-D-galactose	1.8	1.1	0.9	0	0	0.2	0	0
N-Acetylneuraminic acid	0	0	0.3	0	1.0	1.0	2.0	2.0

before conclusions could be drawn from investigations on such glycoproteins, it was necessary to establish that the potential for synthesizing the oligosaccharide units did not vary during the course of the malignancy. Accordingly the myeloma IgA was isolated from patient Bra on two occasions at an interval of 18 months, and the two preparations were found to have identical carbohydrate analyses within the limits of experimental error. Both of these IgA preparations exhibited the polymer-type heterogeneity that is characteristic of IgA, and, since it is possible that there was a variation in carbohydrate content between the various polymeric forms, they were separated by exclusion chromatography and analysed. However, there appeared to be no significant differences in the content of carbohydrate in these fractions.

The points of particular interest in the study of glycoproteins are the character and amount of carbohydrate in the protein, the number of oligosaccharide units in the molecule and their distribution between the various polypeptide chains and, within a particular polypeptide chain, the type of linkage

between carbohydrate and protein, and finally the structure of the oligosaccharide units (Clamp & Putnam, 1967).

The IgA contained, for a molecular weight of 170 000, 2 residues of 6-deoxy-L-galactose, 14–15 of D-mannose, 12–13 of D-galactose, 12–13 of 2-acetamido-2-deoxy-D-glucose, 6 of 2-acetamido-2-deoxy-D-galactose and 5 of N-acetylneuraminic acid. The monosaccharides were identified by both paper and gas-liquid chromatography; this is the first report of the presence of 2-acetamido-2-deoxy-galactose in immunoglobulins. All the carbohydrate was shown to be associated with the heavy chain, as has been previously reported for IgG and IgM (Chaplin, Cohen & Press, 1965).

Glycopeptides were prepared after digestion of the immunoglobulin with Pronase. However, activities other than proteolytic have been attributed to this enzyme, such as the cleavage of the oligosaccharide-protein linkage in orosomucoid (Kamiyama & Schmid, 1962), the partial liberation of sialic acid (Izumi, Makino & Yamashina, 1962) and the partial cleavage of O-glycosidic linkages

(Carubelli, Bhavanandan & Gottschalk, 1965). Therefore the Pronase digest was examined at intervals by gas-liquid chromatography, but no monosaccharides were found. In addition, the use of crystalline pepsin and trypsin produced a comparable range of glycopeptides. The presence of sialic acid did not appear to affect the activity of Pronase, in contrast with the findings of Kamiyama & Schmid (1962), Izumi, Makino & Yamashina (1961) and Eylar (1962), since glycopeptides were isolated with high contents of sialic acid that contained aspartic acid as the sole amino acid. Some preparations of Pronase were found to contain a small amount (1%) of D-glucose, D-mannose and sometimes D-galactose, as has been noted by Jamieson (1965). However, this material came after the glycopeptide peaks during exclusion chromatography and could therefore be separated. Glycopeptide material was obtained in 74% overall yield after two Pronase digests and subsequent exclusion chromatography steps, and the proportions of the monosaccharides were the same as in the original protein and in the derived heavy chain.

The isolated glycopeptides showed two kinds of heterogeneity, which have been termed central and peripheral (Clamp, Dawson & Spragg, 1968). In central heterogeneity the glycopeptides have totally different structures, whereas in peripheral heterogeneity the fundamental or core structure is the same but there are small differences in the terminal residues. In the IgA there were three types of core oligosaccharide units, glycopeptides 1 and 4 forming one type, glycopeptides 2, 5, 6, 7, 8, 9, 10, 11 and 12 forming another and glycopeptide 3 forming the third. Each of these types showed a peripheral heterogeneity, i.e. a variation in certain terminal non-reducing monosaccharides that partially accounted for the large number of glycopeptides found on electrophoresis. The first type, of which glycopeptide 1 and 4 were the only examples, contained D-galactose, 2-acetamido-2-deoxy-D-galactose, serine, threonine and proline in the proportions 3:3:4:4:5 with an additional residue of *N*-acetylneuraminic acid in glycopeptide 4. The carbohydrate-protein linkage involved 2-acetamido-2-deoxy-D-galactose, *O*-glycosidically linked to the hydroxy amino acid serine. This type of linkage is cleaved by a β -elimination reaction under alkaline conditions, and this led to a spurious result during the Morgan & Elson (1934) procedure for determination of liberated acetamidohexose. The size of the glycopeptide was determined by molecular-weight studies on glycopeptide 1 and from the sialic acid content of glycopeptide 4, which migrated to a position corresponding to a glycopeptide with a single sialic acid residue. One-third of the 2-acetamido-2-deoxy-D-galactose was lost during the β -elimination reaction and after

periodate oxidation, and one-third of the D-galactose residues were liberated by β -galactosidase, thereby supporting the presence of 3 residues of each monosaccharide in the original glycopeptide. Periodate oxidation of glycopeptide 1 destroyed all the D-galactose and 1 out of 3 2-acetamido-2-deoxy-D-galactose residues. The acetamidohexose that was oxidized must therefore have been unsubstituted at the C-3 and C-4 hydroxyl groups, but was probably not terminal, since none was released by the β -*N*-acetylhexosaminidase preparation. This acetamidohexose residue must therefore have been substituted at C-6 only. Periodate oxidation of glycopeptide 4 destroyed all the *N*-acetylneuraminic acid and the same amount of acetamidohexose as in glycopeptide 1, but one of the D-galactose residues remained unoxidized. This indicates that in glycopeptide 4 the *N*-acetylneuraminic acid was linked to the C-3 hydroxyl group of the D-galactose residue that was terminal in glycopeptide 1. This type of linkage has been demonstrated in sialolactose (Kuhn, 1958). Since only 1 residue of D-galactose could be released enzymically the oligosaccharide units appear to be unbranched, and the terminal D-galactose to be linked to acetamidohexose. The sequence between these residues and the 2-acetamido-2-deoxy-D-galactose involved in the linkage to serine is unknown, but only one of the acetamidohexose residues is not substituted at either C-3 or C-4. These results are compatible with many structures, but for the purpose of discussion we propose the tentative sequence (I) for glycopeptide 4. Glycopeptide 1 would be similar apart from the absence of the terminal *N*-acetylneuraminic acid.

(I) NANA, *N*-acetylneuraminic acid; GalNAc, *N*-acetylgalactosamine.

Glycopeptides 2, 5, 6, 7, 8, 9, 10, 11 and 12, which together represent the second type of core glycopeptide, all appeared to have a similar fundamental structure consisting of D-mannose, D-galactose and 2-acetamido-2-deoxy-D-glucose in the proportions 3:2:3 relative to 1 residue of aspartic acid, and associated with this structure were 0, 1, 2 or 3 residues of sialic acid, 0-1 residue of 6-deoxy-L-galactose and 0-1 residue of 2-acetamido-2-deoxy-D-glucose. Glycopeptides 7, 10 and 12 stained brown with ninhydrin and contained aspartic acid as the only amino acid. The anodic mobility of these three glycopeptides was directly proportional to their contents of sialic acid, which were 1, 2 and 3 residues respectively. The neutral member of this series (i.e. containing no sialic acid) presumably co-migrated with glycopeptide 3, which also stained

brown with ninhydrin. The remaining glycopeptides contained other amino acids in addition to aspartic acid and stained purple with ninhydrin. The reason for the differences in electrophoretic mobility between glycopeptides containing similar amounts of sialic acid may be related to their amino acid content. Thus in the series glycopeptides 8, 9 and 10, glycopeptide 10 contained only aspartic acid and had the highest mobility, whereas glycopeptide 8 contained in addition 1 residue of valine and was the slowest of the series. The presence of less hydrophobic amino acid residues in glycopeptide 9 would account for its intermediate migration. Similar explanations may account for the different electrophoretic mobilities of the series glycopeptides 5, 6 and 7. The sialic acid was shown to be terminal in glycopeptides 7, 8 and 10 by its rapid release with neuraminidase and after dilute acid hydrolysis, and was identified as *N*-acetylneuraminic acid by gas-liquid chromatography. Subsequent incubation of glycopeptides 8 and 10 with β -galactosidase liberated D-galactose. All the D-galactose in the neutral glycopeptide 2 was susceptible to D-galactose oxidase. Since in these glycopeptides all the sialic acid and D-galactose was destroyed by periodate, sialic acid must have been linked to D-galactose at C-2, as in human IgG (Rothfus & Smith, 1963), at C-4, as in guinea-pig serum glycoproteins (Simkin, Seshadri & Skinner, 1964) or at C-6. Glycopeptides 11 and 12 contained 3 residues of *N*-acetylneuraminic acid and only 2 residues of D-galactose, suggesting either that 2 sialic acid residues were linked to a single D-galactose residue or that 2 sialic acid residues were linked together, as in disialolactose (Kuhn & Gauhe, 1965). All the 6-deoxy-L-galactose was oxidized by periodate, which is compatible with a terminal position, as is the variability of the 6-deoxy-L-galactose content in this series of glycopeptides, which was independent of the other monosaccharides. The 6-deoxy-L-galactose did not appear to be linked to D-galactose, since in glycopeptide 2 all the D-galactose was susceptible to D-galactose oxidase. Further, this glycopeptide appeared to have the same fundamental structure as the other glycopeptides of this type, which contained significant amounts of 6-deoxy-L-galactose in addition to sialic acid residues. The sialic acid in these glycopeptides was linked to D-galactose, which was unlikely to have other substituents since all the D-galactose was destroyed by periodate. About 3 moles of 2-acetamido-2-deoxy-D-glucose/mole remained after oxidation of glycopeptides 5, 6, 7, 8, 9 and 10 and in Sephadex fraction 3, suggesting that the acetamidohexose in excess of 3 residues was in a terminal position. This was subsequently confirmed with β -*N*-acetylhexosaminidase. Comparison of the periodate-oxidation results before

and after enzymic removal of this 2-acetamido-2-deoxy-D-glucose showed that the number of surviving D-mannose residues decreased from 1.5 to 1.0. These findings would be consistent with the attachment of the terminal 2-acetamido-2-deoxy-D-glucose to a D-mannose residue already substituted at C-2 or C-4. Dilute acid hydrolysis provided further evidence for the sequence sialic acid $\rightarrow$ D-galactose $\rightarrow$ 2-acetamido-2-deoxy-D-glucose, as reported in orosomucoid (Eylar & Jeanloz, 1962; Barker, Pardoe, Stacey & Hopton, 1963), fetuin (Spiro, 1962), ovomucoid (Montreuil, Adam-Chossou & Spik, 1965) and a nucleotide-oligosaccharide complex from goat colostrum (Jourdian, Shimizu & Roseman, 1961). No D-mannose was released during this procedure, indicating a more internal location for this monosaccharide. Synthetic 2-acetamido-1-N-(4'-L-aspartyl)-2-deoxy- β -D-glucopyranosylamine was used as a model compound for the carbohydrate-amino acid linkages in these glycopeptides. This compound stained brown with ninhydrin and had an electrophoretic mobility different from the alternative compound 2-acetamido-1-N-(1'-L-aspartyl)-2-deoxy- β -D-glucopyranosylamine, which stained purple with ninhydrin. All the glycopeptides from the IgA (glycopeptides 12, 10, 7 and 3) and from ovalbumin, which contained aspartic acid as the sole amino acid, stained brown with ninhydrin, and this colour may therefore indicate the presence of a similar linkage. An enzyme that hydrolyses the synthetic glycopeptide has been reported in ovine epididymis (Murakami & Eylar, 1965) and a similar enzyme was shown (Clamp, Dawson, Hough & Khan, 1966) to be present in the β -N-acetylglucosaminidase preparation used in these studies. The enzyme liberated aspartic acid from an ovalbumin glycopeptide preparation and from glycopeptide 10, providing further evidence for this linkage. However, when other amino acids in addition to aspartic acid were present, the glycopeptide appeared to be resistant to the enzyme.

The tentative structure (II) is proposed for this series of glycopeptides.

(II) NANA, *N*-acetylneuraminic acid; GlcNAc, *N*-acetylglucosamine; Fuc, fucose.

Table 12. *Oligosaccharide units of the IgA*

The results are expressed as residues of monosaccharide in each glycopeptide corrected to the nearest whole number. The average values for each type are given in parentheses. The theoretical value is the sum of the residues in the postulated oligosaccharide units.

Type of oligosaccharide unit ... No. of units Monosaccharide	No. of monosaccharide residues			Total residues	
	1	2	3	Theoretical	Original protein
	2	3	1		
6-Deoxy-L-galactose	0	0-1 (0.7)	0	2	2
D-Mannose	0	3	4	13	14-15
D-Galactose	3	2	0	12	12-13
2-Acetamido-2-deoxy-D-glucose	0	3-4 (3.3)	3	13	12-13
2-Acetamido-2-deoxy-D-galactose	3	0	0	6	6
N-Acetylneuraminic acid	0	0-3 (1.7)	0	5	5

Thus the glycoprotein is unusual in that two distinct types of carbohydrate-amino acid linkage are present, namely an *O*-glycosidic linkage between 2-acetamido-2-deoxy-D-galactose and L-serine and an *N*-glycosidic linkage between 2-acetamido-2-deoxy-D-glucose and L-aspartic acid. The presence of two different types of carbohydrate-protein linkage has been reported in lactotransferrin (Spik, Monsigny & Montreuil, 1966) and renal glomerular basement membrane (Spiro, 1967), but different monosaccharides and amino acids were involved in the *O*-glycosidic linkages.

The third type of core glycopeptide was represented by glycopeptide 3, which stained brown with ninhydrin and contained 6-deoxy-L-galactose, D-mannose, D-galactose, 2-acetamido-2-deoxy-D-glucose and aspartic acid in the proportions 0.1:3.9:0.6:3.2:1.0. Thus the D-mannose content was higher and the D-galactose content lower than in the type 2 glycopeptides. The small amount of 6-deoxy-L-galactose and D-galactose may have been due to contamination from a glycopeptide of the second type containing 6-deoxy-L-galactose, D-mannose, D-galactose and 2-acetamido-2-deoxy-D-glucose in the proportions 0.3:3.0:2.0:3.5. The presence of this neutral glycopeptide would complete the series glycopeptides 12, 10 and 7, which all stained brown with ninhydrin, have aspartic acid as the sole amino acid and contain 3, 2 and 1 residues of sialic acid respectively. If this glycopeptide represented 30% by weight of glycopeptide 3, this would give the observed analysis and would explain the low results obtained in some studies. The glycopeptide of the third type would then contain only D-mannose and 2-acetamido-2-deoxy-D-glucose in the ratio 4:3, which is similar to glycopeptides found in IgM (J. R. Clamp, G. Dawson & B. P. Spragg, unpublished work). The

absence of a terminal non-reducing 2-acetamido-2-deoxy-D-glucose residue was shown by periodate oxidation and β -N-acetylglucosaminidase. D-Mannose appeared to be terminal and to be present in a sequence of at least 3 D-mannose residues, since incubation with emulsin rapidly liberated over 90% of the D-mannose with the concomitant release of only 1.5 residues of 2-acetamido-2-deoxy-D-glucose. After periodate oxidation, all the acetamidohexose survived, together with 3 out of 4 D-mannose residues, showing that the glycopeptide was unbranched, that the D-mannose units were substituted at C-3 and that the 2-acetamido-2-deoxy-D-glucose residues were substituted at either C-3 or C-4. The only amino acid present was aspartic acid, suggesting a similar linkage to that in the type 2 glycopeptides.

The tentative structure (III) is therefore proposed for the type 3 glycopeptide.

From the yields and analyses of these glycopeptides in comparison with the original glycoprotein there would appear to be an average of two type 1, three type 2 and one type 3 oligosaccharide units associated with each molecule of IgA (Table 12). The distribution of the oligosaccharide units on the heavy chains must therefore be asymmetrical. Analyses of the larger glycopeptides isolated after digestion of the protein with pepsin and trypsin suggest that the oligosaccharide units are present in the same region of the heavy chain.

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