

Structure of the Carbohydrate Units of IgE Immunoglobulin

I. OVER-ALL COMPOSITION, GLYCOPEPTIDE ISOLATION, AND STRUCTURE OF THE HIGH MANNOSE OLIGOSACCHARIDE UNIT*

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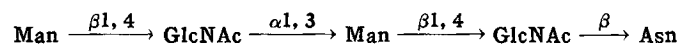
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SUMMARY

The total carbohydrate composition of an IgE myeloma protein has been determined. Mannose, *N*-acetylglucosamine, galactose, sialic acid, and fucose were found to be present in the amount of 16, 12, 7, 5, and 4 moles, respectively, per mole of heavy chain. Previously it had been determined that all of the carbohydrate moieties were located on the heavy chain of the IgE protein (KOCHWA, S., TERRY, W. D., CAPRA, J. D., AND YANG, N. L. (1971) *Ann. N. Y. Acad. Sci.* 190, 49).

Five major glycopeptide-containing fractions were isolated after pronase degradation of the protein. The type designated B contained sialic acid, fucose, mannose, galactose, and *N*-acetylglucosamine in the molar ratios of 1 to 2:1:3:2:4. Three of the B-type oligosaccharide units were found to be present per heavy chain. The complete structure of these glycopeptides is presented in the following paper (BAENZIGER, J., KOCHWA, S., AND KORNFELD, S. (1974) *J. Biol. Chem.* 249, 1897). In addition, a high mannose glycopeptide, termed C-1, was isolated which contained only mannose and *N*-acetylglucosamine in the ratio of 6:2. Only one chain of this type was present per heavy chain. The complete sequence of this glycopeptide was determined using a combination of sequential enzymatic degradation, periodate oxidation, and methylation. After removal of 4 of the 6 mannose residues with α -mannosidase, a core with the following sequence remained.



This is the first example of an α -linked *N*-acetylglucosamine to be described in a circulating glycoprotein. This glycopeptide is also unusual in that it does not have the more commonly found chitobiose core unit ($\text{GlcNAc} \xrightarrow{\beta 1, 4}$

$\text{GlcNAc} \xrightarrow{\beta} \text{Asn}$) and gives no evidence of microheterogeneity.

A new class of immunoglobulins designated IgE was described by Ishizaka and Ishizaka in 1966 (1). These authors also showed that this immunoglobulin type possessed reagenic antibody activity. The following year the first patient exhibiting a myeloma protein of the IgE immunoglobulin class was reported (2), and in 1969 the present patient (P. S.) was found (3). Since IgE is present in such small amounts in normal serum (6 to 780 ng per ml) (4), these myeloma proteins have offered a unique opportunity for biochemical characterization of IgE immunoglobulin.

Our laboratory has been interested in determining the structures of the various oligosaccharide units found in the different classes of immunoglobulins. Structures have been proposed by us and others for the oligosaccharide units of IgG (5, 6), IgM (7, 8), and IgA¹ (9, 10), but no information aside from gross composition (11, 12) has been available regarding IgE. Since each of the immunoglobulin classes appear to have different properties and functions, it is important to determine whether the carbohydrate structures differ from one class to another, as this would make available the potential for unique functional roles for the different oligosaccharide structures.

In this report we present data on the total carbohydrate composition of an IgE myeloma protein and describe the complete sequence of one of the oligosaccharide units of the molecule. The following paper is concerned with the structure of the other oligosaccharide units of IgE (13).

EXPERIMENTAL PROCEDURE

Preparation and Isolation of IgE Glycopeptides—IgE (518 mg), isolated from Patient P. S. as described previously (14), was digested with 1% (w/w) Pronase in 0.1 M Tris buffer, pH 7.9,

¹ J. Baenziger and S. Kornfeld, manuscript in preparation.

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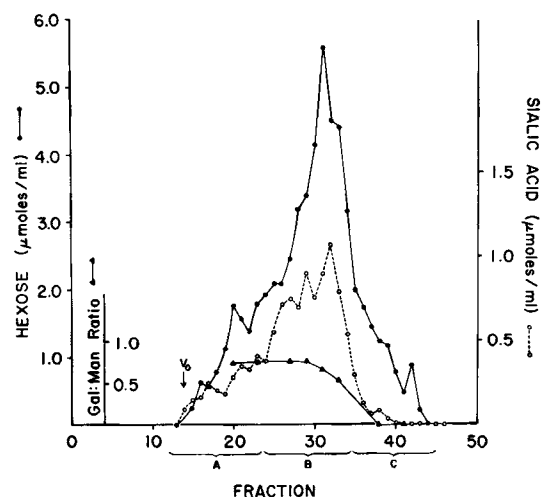


FIG. 1. Gel filtration of pronase digest on Bio-Gel P-10. The column (1.6×83 cm) was eluted with H_2O and each fraction (6.3 ml) analyzed for sialic acid and hexose. The indicated fractions were subjected to total carbohydrate analysis by gas-liquid chromatography and the ratio of galactose to mannose computed. Fractions 14 to 24 and 25 to 35, containing both galactose and mannose, were combined into two pools. Fractions 36 to 44, containing only mannose, were also pooled. Sialic acid, $\circ$ --- $\circ$; hexose, $\bullet$ — $\bullet$.

containing 0.002 M $CaCl_2$ at 37° under a toluene atmosphere. After 24 hours the pH was adjusted to 7.9 with NaOH and additional Pronase (1.5% w/w) was added. Twenty-four hours later the digest was centrifuged to remove precipitated material and the supernatant applied to a Sephadex G-25 column (3.5×80 cm). The column was eluted with H_2O , and those fractions containing hexose as detected by the phenol- H_2SO_4 method (15) were combined and again subjected to Pronase digestion (1% w/w) for 48 hours. The product of this second digestion was applied to a Bio-Gel P-10 column (1.6×83 cm) and eluted with H_2O . A broad peak containing hexose and sialic acid emerged with the void volume (Fig. 1). Individual fractions were assayed for total sugar content by gas-liquid chromatography and the ratio of galactose to mannose was plotted (Fig. 1). There were at least two populations of glycopeptides in this digest, since the fractions designated A and B (Fig. 1) contained galactose, mannose, and sialic acid, while those designated C contained mannose, trace amounts of sialic acid, and virtually no galactose. The fractions were pooled as indicated in Fig. 1 and subjected to ion exchange chromatography on DEAE-cellulose (Fig. 2) which yielded five major glycopeptides, B-1, B-2, B-3, B-4, and C-1. It seems likely that A-2, A-3, and A-4 correspond to B-2, B-3, and B-4, respectively, since they have virtually identical compositions.

Analytical Methods—The methods for carbohydrate analyses, with the exception of the gas-liquid chromatographic technique, have been described previously (5). Gas-liquid chromatographic analysis of carbohydrates was carried out by a modification of the method of Reinhold (16) in which the samples were hydrolyzed for 4 hours in 1.5 N methanolic HCl at 95° . The *O*-trimethylsilyl ether derivatives were separated on columns of 3% Se-30 on Gas-chrom Q and 3% Ov-17 on Gas-chrom Q (Applied Science Labs) using an F & M 402 gas chromatograph equipped with dual columns and dual recorder, programming from 120 – 250° at 3° per min. The columns used were glass (2.0 mm inside diameter $\times$ 88 cm) and a gas flow of 15 to 25 cc per min was maintained for the helium carrier. Arabinol was

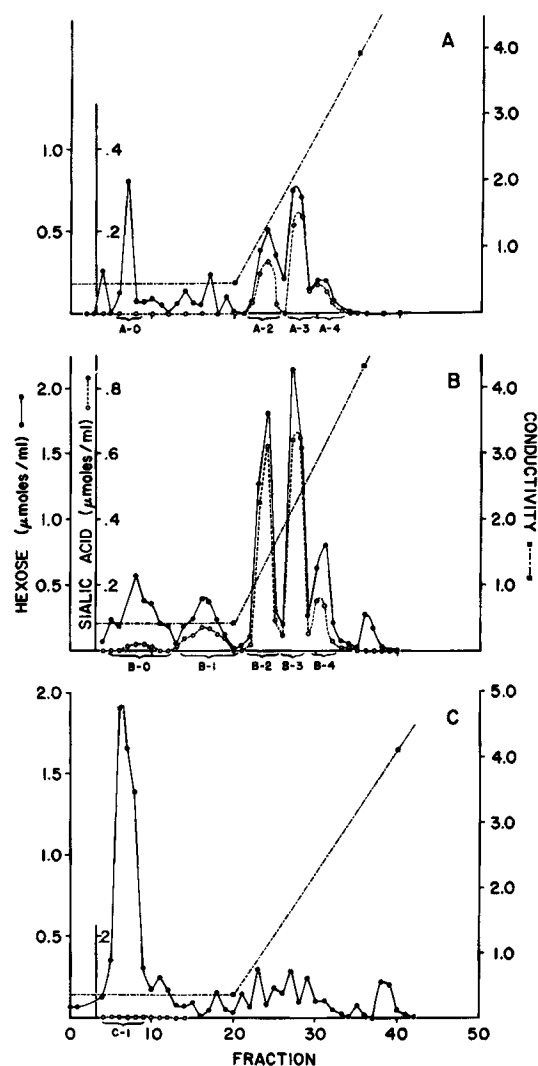


FIG. 2. DEAE-cellulose chromatography of the glycopeptide-containing fractions from the Bio-Gel P-10 column. Glycopeptide Fractions A, B, and C from the Bio-Gel P-10 column were loaded on DEAE-cellulose columns (1.5×20 cm) which were equilibrated with 4 mM phosphate buffer, pH 6.8. After washing the columns with 80 ml of the starting buffer, elution was carried out with a linear gradient (100 ml each) of 4 to 100 mM phosphate buffer, pH 6.8. Each 6-ml fraction was analyzed for sialic acid ($\circ$ --- $\circ$) and hexose ($\bullet$ — $\bullet$). Fractions were then pooled as noted.

used as the internal standard and areas were computed by triangulation. Glycopeptides which contained asparagine as their only amino acid residue displayed some degree of resistance to complete hydrolysis of the *N*-acetylglucosamine residues in the core when the hydrolysis conditions described by Reinhold (16) or those mentioned above were used. Greater release and more consistent results were obtained with such glycopeptides using 4 N aqueous HCl at 100° for 4 hours in evacuated tubes.

Methods for amino acid determination and sequential enzymatic degradation of the glycopeptides have been described previously (5).

Molecular Weight Determinations—Estimates of the molecular weight of each glycopeptide were made by gel filtration on Sephadex G-50 as described by Bhatti and Clamp (17).

Methylation Analysis—Glycopeptides were methylated by the method of Hakomori (18) as described by Björndahl and Lundblad (19). The preparation, separation, and identification of the alditol acetate derivatives of the methylated neutral sugars

TABLE I
Glycosidase activity present in enzyme preparations used for sequential degradations

Enzyme Preparation	Glycosidase Activity (units per ml)					
	α -Galactosidase	β -Galactosidase	α -Mannosidase	β -Mannosidase	α -N-Acetylglucosaminidase	β -N-Acetylglucosaminidase
α -N-Acetylglucosaminidase - Pig Liver	< 0.02	< 0.07	< 0.04	< 0.02	3.66	< 0.09
β -N-Acetylglucosaminidase - Jack Bean	----	< 0.001	< 0.061	----	< 0.001	55.0
β -Galactosidase - <i>E. coli</i>	< 0.001	9700	----	----	----	----
β -Galactosidase - Jack Bean	2.0	66.0	< 0.03	----	----	< 0.06
α -Galactosidase - Ficin	19.4	< 0.01	< 0.01	----	----	4.85
α -Mannosidase - Jack Bean	----	----	46.6	< 0.002	----	< .001
β -Mannosidase - Bromelain	0.31	0.49	< 0.03	0.29	----	0.08
Rat Epididymal Preparation I	< 0.002	11.0	< 0.002	35.5	< 0.006	70.0
" " II	----	----	< 0.0005	11.7	< 0.001	----

One unit of enzyme activity is defined as the amount of enzyme which releases 1 mmole of substrate in 1 hour at 37° C. All of the enzymes except *E. coli* β -galactosidase and bromelain β -mannosidase were incubated in .05 M Na citrate buffer, pH 4.6, containing the appropriate p-nitrophenylglycoside: .003 M p-NO₂ α -D-galactopyranoside, .003 M p-NO₂ β -D-galactopyranoside, .001 M p-NO₂ β -D-N-acetylglucosamine, .01 M p-NO₂ β -D-mannopyranoside, .005 M UDP-N-acetyl-D-glucosamine. β -mannosidase activities from bromelain were assayed in .05 M glycine buffer, pH 3.5, *E. coli* β -galactosidase was assayed in .1 M NaPO₄ buffer, pH 7.0.

by gas-liquid chromatography and mass spectrometry were done as described previously (5). In addition, permethylated glycopeptides C-1 and B-2 were subjected to acetolysis with 0.5 M H₂SO₄ in 95% acetic acid, reduction, and acetylation as described by Stellner *et al.* (20). The partially methylated additol acetates and 2-deoxy-2-*N*-methylacetamidohexitol acetates were separated and identified by gas chromatography and mass spectrometry as described by Stellner *et al.* (20).

Periodate Oxidation—Samples were subjected to periodate oxidation in 50 to 100 μ l of 0.05 M acetate buffer, pH 4.6, containing 0.08 M NaIO₄ for 12 to 16 hours at 4° in the dark. The reaction was terminated by addition of 1 to 2 μ l of ethylene glycol followed by incubation at room temperature for 1 hour. Arabitol was then added as the internal standard and the samples thoroughly dried before hydrolysis in either 1.5 N methanolic HCl for gas-liquid chromatography or 4 N aqueous HCl.

Enzymes—Neuraminidase (EC 3.2.1.18) from *Vibrio cholerae* and Pronase were purchased from Calbiochem. β -Galactosidase (EC 3.2.1.30) and α -mannosidase (EC 3.2.1.24) from jack bean meal were prepared as described previously (21). An enzyme preparation containing the activities listed in Table I was prepared from frozen rat epididymides (Pel-Freez) by a modification of the method of Levvy and Conchie (22) as described previously (5). α -Galactosidase was prepared from ficin (National Biochemicals Corp., Cleveland, Ohio) as described by Li and Li (23). In addition to the β -mannosidase from rat epididymus, a preparation was made from pineapple stem bromelain (NBC) as described by Li and Lee (24). α -*N*-Acetylglucosaminidase, completely free of β -*N*-acetylglucosaminidase activity, was prepared by a modification of the method of Weissmann (25) using UDP-*N*-acetylglucosamine as the substrate. The enzyme was precipitated with ammonium sulfate as described (25) and passed over successive columns of Cellex T (Bio-Rad) in 0.005 M Tris containing 0.086 M citrate, pH 7.5, and Cellex T in 0.0025 M Tris containing 0.043 M citrate, pH 8.0. The enzyme activity was not adsorbed by either column and was precipitated from the effluent of the second Cellex T column by addition of solid (NH₄)₂SO₄ to 31% saturation. Gel filtration of the enzyme on Sephadex G-150 equilibrated in 0.01 M citrate buffer, pH 6.0, yielded a single protein peak with coincident α -*N*-acetylglucosaminidase activity. The fractions containing enzyme activity were stored at -20°.

Escherichia coli β -galactosidase (EC 3.2.1.23) was purchased from Worthington Biochemicals and digests were carried out as described by Slomiany and Meyer (26) scaled down to 150- to 200- μ l incubation mixtures. The data in Table I summarize the various activities of the different glycosidase preparations. In addition, β -aspartyl-*N*-acetylglucosamine amidohydrolase was purified from hen oviduct as described by Tarentino and Maley (27). Carboxypeptidase A (bovine) and carboxypeptidase B (hog) were purchased from Sigma.

RESULTS

Composition of IgE and of Glycopeptides Isolated from IgE—The carbohydrate and amino acid compositions of intact IgE and of the respective glycopeptides isolated from IgE are given in Tables II and III. The compositions of B-1, B-2, B-3, and B-4 are presented as residues per glycopeptide molecule with mannose being set at 3, while C-1 is presented as residues with *N*-acetylglucosamine set at 2. Since A-2, A-3, and A-4 have the same composition as B-2, B-3, and B-4, respectively (data not shown), and are probably derived from the same oligosac-

TABLE II
Carbohydrate composition of IgE immunoglobulin

Carbohydrate residue	Found	Predicted ^a
	moles/mole heavy chain $\pm$ S.D.	
Mannose	15.7 $\pm$ 1.8	15
<i>N</i> -Acetylglucosamine	12.3 $\pm$ 1.8	14
Galactose	6.9 $\pm$ 0.5	6
Sialic acid	5.4 $\pm$ 0.2	5-6
Fucose	3.6 $\pm$ 0.4	3

^a Predicted on the basis of 1 oligosaccharide unit of the C type and 3 of the B type per heavy chain (see Table III). The standard deviations (S.D.) were calculated on the basis of five or more determinations.

charide chains on the intact IgE molecule, the actual yield of these glycopeptides is probably higher than indicated in Table III. The carbohydrate composition of intact IgE is presented as moles of sugar per mole of heavy chain, since it was previously determined that only the heavy chain contained carbohydrate (14). Based on the recoveries of the various glycopeptides and the actual composition of the IgE heavy chain, it appears that there is one glycopeptide of the C type and three of the B type per heavy chain. This would give the predicted composition noted in Table II, which agrees extremely well with the values obtained for the intact IgE molecule; however, the possible presence of a fourth chain of the B type cannot be completely excluded.

Structural Studies of Glycopeptide C-1—As indicated in Table III, glycopeptide C-1 contains mannose and *N*-acetylglucosamine in the ratio of 6:2 and approximately 9 amino acid residues. Assuming nine amino acids are present, the calculated molecular weight of 2350 is in good agreement with the value of 1910 estimated by exclusion chromatography (Fig. 3 and Table IV) since the nine amino acids might not be expected to contribute their full calculated molecular weight on a column calibrated with oligosaccharides (17). Removal of these amino acids with carboxypeptidase A and B followed by treatment with β -aspartyl-*N*-acetylglucosamine amidohydrolase resulted in an oligosaccharide which when treated with NaBH₄ yielded *N*-acetylglucosaminitol, indicating a β linkage of the *N*-acetylglucosamine to the asparagine.

In order to determine the sequence of the sugars of the oligosaccharide chain, a series of enzymatic and periodate degradation steps were performed as outlined in Fig. 4. Table V summarizes the data obtained from these steps and Fig. 5 shows representative gas-liquid chromatographic scans from which some of the data for Table V were obtained. In addition, methylation analyses of the glycopeptide were performed. The proposed structure of glycopeptide C-1 is also presented in Fig. 4.

The results of the methylation studies are presented in Table VI and indicate that 3 of the 6 mannose residues are terminal (2,3,4,6-tetramethylmannose) while neither of the *N*-acetylglucosamine residues is terminal. The methylation pattern is in good agreement with the finding that both *N*-acetylglucosamine residues and 2 of the 6 mannose residues are resistant to destruction by sodium metaperiodate (Table V, Experiment 1).²

² Since sodium metaperiodate oxidizes sugars with unsubstituted vicinal hydroxyl groups, the mannose residues which give rise to the 2,4-dimethyl derivatives and the *N*-acetylglucosamine residues would be expected to be periodate-resistant. The other 4 mannose residues should be periodate-sensitive.

TABLE III
Carbohydrate and amino acid composition of IgE glycopeptides

Glycopeptide	B-1	B-2	B-3	B-4	C-1
Yield (moles/mole heavy chain)	0.40	0.61	0.61	0.23	0.54
Residues ^a					
Mannose	3.0 ^b	3.0 ^b	3.0 ^b	3.0 ^b	6.0 ± 0.1
N-Acetylglucosamine	4.2 ± 0.4	4.0 ± 0.6	3.9 ± 0.5	4.2 ± 0.1	2.0 ^c
Galactose	2.0 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.2 ± 0.3	0
Sialic Acid	1.1 ± 0.1	2.0 ± 0.2	2.0 ± 0.1	2.0 ± 0.3	< 0.2
Fucose	1.0 ± 0.1	1.0 ± 0.2	1.1 ± 0.1	0.8 ± 0.1	0
Asparagine	0.5	1.3	0.6	1.0	1.1
Threonine	0.2	0	0.3	0	1.4
Serine	0	0.1	0	0	0.8
Glutamic Acid	0	0	0	0.3	0.3
Proline	0	0	0	0	2.5
Glycine	0.2	0.1	0.3	0	1.6
Alanine	0.3	0.1	0.3	0	0.6
Lysine	0.1	0.2	0	0	1.3
CM-Cysteine	0	0	0	N.D.	N.D.

^a The residues are expressed as moles of sugar per mole glycopeptide ± the standard deviation.

^b Mannose set equal to three

^c N-acetylglucosamine set equal to two

N.D. - Not determined

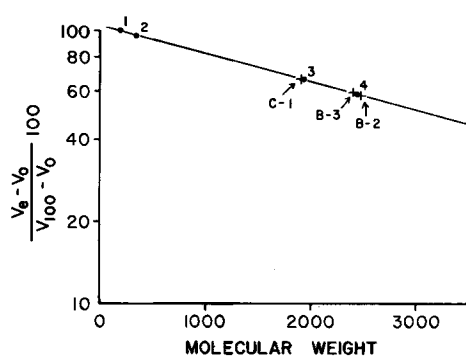


FIG. 3. Estimation of molecular weights of the IgE immunoglobulin glycopeptides by exclusion chromatography on Sephadex G-50-80. Samples were applied to a column (1.8 × 85 cm) of Sephadex G-50-80 and eluted with 0.9% NaCl and 0.01 M NaHCO₃. Fractions of 3.1 ml were collected. The applied samples contained, in addition to the IgE glycopeptides, the following materials: blue dextran to locate the void volume (V₀), [¹⁴C]mannose to determine V₁₀₀, an IgG glycopeptide (mol wt 1919), and an IgA glycopeptide (mol wt 2440). The IgG and IgA glycopeptides had been labeled by acetylation with [³H]acetic anhydride. The compounds used for calibration were: 1, mannose; 2, lactose; 3, IgG glycopeptide (mol wt 1919); and 4, IgA glycopeptide (mol wt 2440).

TABLE IV

Calculated and estimated molecular weights of IgE glycopeptides

The calculated molecular weights were determined from the compositions of the glycopeptides shown in Table III. The estimated molecular weights were determined by exclusion chromatography as described in Fig. 3.

Glycopeptide	Calculated mol wt	Estimated mol wt
C-1	2350 ^a	1910
B-2	2441	2460
B-3	2441	2340

^a Assuming nine amino acids are present.

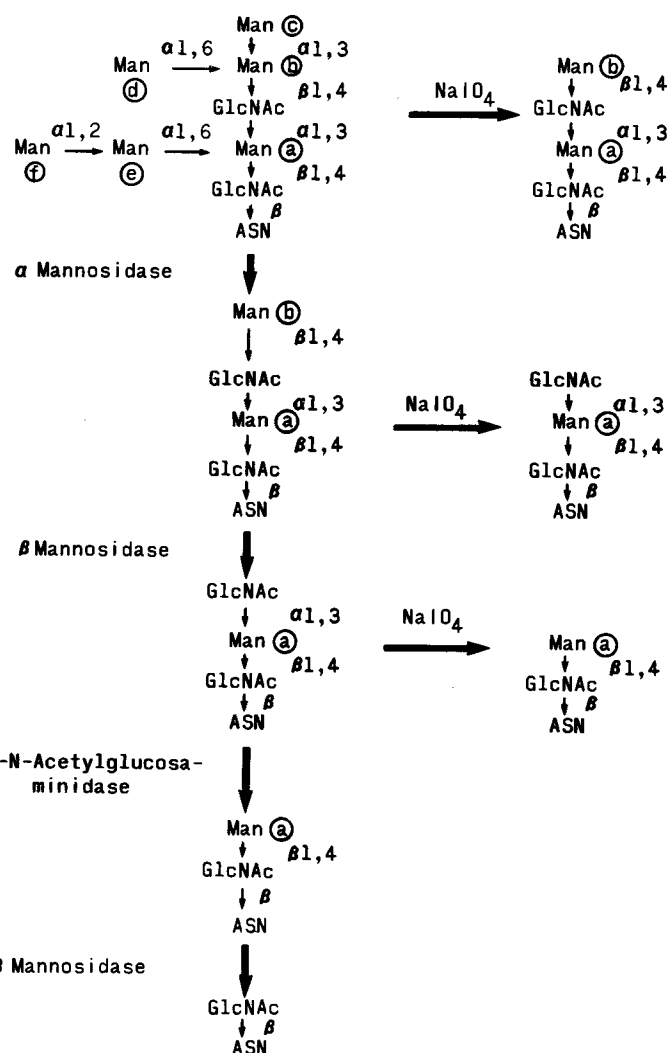
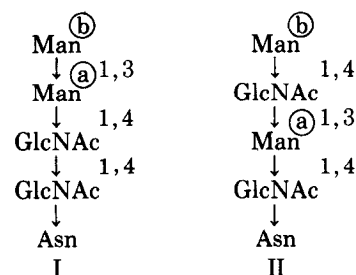


FIG. 4. Scheme for characterization of C-1 glycopeptide of IgE.

As shown in Table V, Experiment 2, treatment of the intact glycopeptide with β -mannosidase isolated from pineapple stem bromelain failed to release any mannose, whereas treatment with α -mannosidase led to the rapid release of 4 residues of mannose (residues **c**, **d**, **f**, and **e** in Fig. 4). When the core remaining after α -mannosidase treatment was subjected to periodate oxidation, 1 of the 2 mannose residues was destroyed and both *N*-acetylglucosamine residues survived (Table V, Experiment 3), indicating that neither *N*-acetylglucosamine residue is terminal in the core and that the surviving mannose (residue **a** in Fig. 4) must be substituted in position 3. On

the basis of these data the core sugars could be arranged in either of the following sequences.



The following experiments prove that the correct structure is Sequence II. After removal of 4 mannose residues with α -mannosidase, 1 of the remaining mannose residues (residue **b**) could be released with β -mannosidase (Table V, Experiments 4 and 5). Further treatment with α -mannosidase and β -*N*-acetylglucosaminidase failed to release any of the residual sugars (Table V, Experiment 4). Periodate treatment of the residual

TABLE V
Effects of glycosidases and periodate oxidation on glycopeptide C-1

Experiment	Residues released or destroyed		Residues remaining ^a	
	Mannose	GlcNAc	Mannose	GlcNAc
Intact Glycopeptide C-1.....			5.97	2.00
1. Periodate oxidation.....	4.07	0	1.90	2.00
2. β -Mannosidase alone.....	<0.15			
Then α -mannosidase.....	3.59			
3. α -Mannosidase alone.....	4.07		1.90	2.00
Then NaIO ₄	1.16	0.07	0.74	1.93
4. α -Mannosidase + β - <i>N</i> -Acetylglucosaminidase.....	4.00	0		
Then β -mannosidase.....	1.07			
Then α -mannosidase + β - <i>N</i> -Acetylglucosaminidase.....	0.03	0		
Then NaIO ₄	0	0.71	1.12	1.29
5. α -Mannosidase.....	4.00			
Then β -mannosidase.....	1.07			
Then α - <i>N</i> -acetylglucosaminidase.....		0.98		
Then α -mannosidase.....	0.09			
Then β -mannosidase.....	0.91			

^a The values shown for residues remaining were determined by direct assay of the reisolated glycopeptides using the gas-liquid chromatographic method. The released mannose was determined enzymatically (5) and the released GlcNAc was determined by the Morgan-Elson colorimetric method (5).

TABLE VI
Relative proportions of methylated sugars in IgE glycopeptide C-1

Proportions were determined by setting the total for mannose to 6.00.

Glycopeptide	Mannose			2- <i>N</i> -Methyl- <i>N</i> -acetylglucosamine
	2,3,4,6-Tetramethyl	3,4,6-Trimethyl	2,4-Dimethyl	3,6-Dimethyl
C-1	2.91	1.07	2.02	2.60

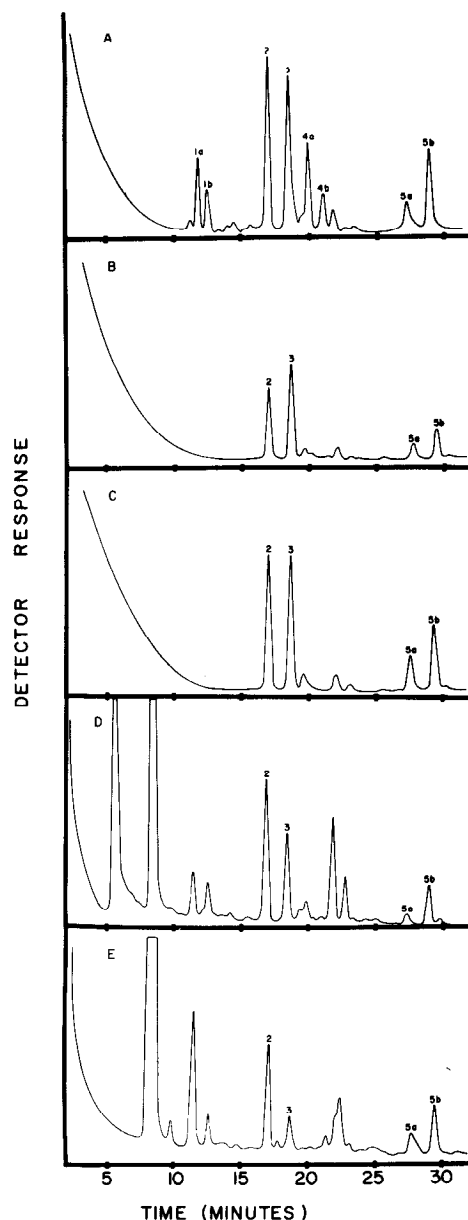
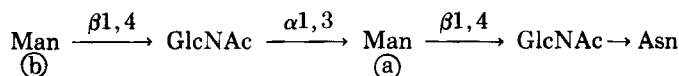


FIG. 5. Gas-liquid chromatographic scans of composite standard (A), intact C-1 (B), sodium metaperiodate-treated C-1 (C), C-1 core reisolated after removal of all α -linked mannose (D), and C-1 core reisolated after removal of all α -linked mannose followed by periodate oxidation (E). In addition to 0.05 μ mole of the internal standard, arabinol, the samples contained: Scan A, 0.05 μ mole each of fucose, mannose, galactose, and *N*-acetylglucosamine; Scan B, 0.015 μ mole of C-1; Scan C, 0.030 μ mole of C-1; Scan D, 0.030 μ mole of C-1; Scan E, 0.030 μ mole of C-1. The numbered peaks represent the following: 1a and 1b, fucose; 2, arabinol; 3, mannose; 4a and 4b, galactose; 5a and 5b, *N*-acetylglucosamine.

core (containing 1 mannose and 2 *N*-acetylglucosamine residues) spared the mannose residue but destroyed 1 of the 2 *N*-acetylglucosamine residues, indicating that removal of the β -linked mannose had resulted in the appearance of a terminal *N*-acetylglucosamine (Table V, Experiment 4). This *N*-acetylglucosamine residue was completely resistant to release by β -*N*-acetylglucosaminidase from three sources but could be quantitatively released by α -*N*-acetylglucosaminidase isolated from pig liver (Table V, Experiment 5). It was then possible to remove the remaining mannose residue with β -mannosidase, but not with α -mannosidase (Table V, Experiment 5). Thus the structure of the core must be



In order to show that the mannose residue $\textcircled{b}$ linked β to the outer *N*-acetylglucosamine gave rise upon methylation to a 2,4-dimethylmannose derivative rather than a 3,4,6-trimethylmannose derivative in the intact glycopeptide, the following experiment was performed. The intact glycopeptide was treated with periodate, then reduced with NaBH_4 and hydrolyzed for 1 hour in 0.1 *N* H_2SO_4 at 80° to release reduced fragments produced by this treatment (28). The resultant products were then separated on a calibrated Bio-Gel P-2 column. No free mannose was found and the residual glycopeptide had a mannose to *N*-acetylglucosamine ratio of 1:1. If the mannose residue designated $\textcircled{e}$ in Fig. 4 (substituted at position 2 only) had been inserted between mannose residue $\textcircled{b}$ and the outer *N*-acetylglucosamine, one would have found 1 residue of free mannose (derived from mannose residue $\textcircled{b}$) and a residual glycopeptide with a mannose to *N*-acetylglucosamine ratio of 0.5:1. This was not the case, proving that mannose residue $\textcircled{b}$ must be disubstituted.

Since the inner mannose residue (residue $\textcircled{a}$) is substituted at position 3 by an *N*-acetylglucosamine residue (resulting in periodate resistance), it must have been substituted at position 6 by 1 of the mannose residues released with α -mannosidase (residue $\textcircled{e}$ in Fig. 4). This is because only the 2 mannose residues originally substituted at positions 3 and 6 could survive periodate oxidation.

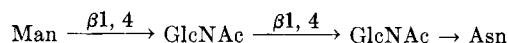
On the basis of these data the structure proposed for glycopeptide C-1 is as shown in Fig. 6. The only residue whose location is not unequivocally established is mannose residue $\textcircled{f}$ which could be linked to either mannose residue $\textcircled{c}$, $\textcircled{d}$, or $\textcircled{e}$.

DISCUSSION

The IgE myeloma protein (P. S.) used in these studies has two basic types of glycopeptide units. There are 3 oligosaccharide units per heavy chain which contain sialic acid, fucose, galactose, mannose, and *N*-acetylglucosamine in the molar ratio of 1 to 2:1:2:3:4 and 1 oligosaccharide unit per heavy chain containing only mannose and *N*-acetylglucosamine (molar ratio

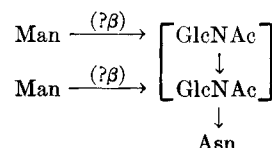
6:2). This distribution agrees well with data on the total composition for the intact IgE and with recoveries of the individual oligosaccharides. IgE (P. S.) appears to be quite similar to the other IgE myeloma protein (N. D.) regarding the relative proportions of each sugar and the absence of *N*-acetylgalactosamine. However, IgE (P. S.) has about 20 to 30% less carbohydrate than IgE (N. D.) (12).

Glycopeptides containing only mannose and *N*-acetylglucosamine residues have been identified in a number of glycoproteins including IgM (7, 8) and IgA myeloma proteins (10). The glycopeptides of this type have displayed some remarkable similarities, especially as regards the core of the molecule. In the case of Taka amylase A (29, 31), bromelain (30, 31), and ovalbumin (31) glycopeptides, the core has been shown to have the following structure:



Bovine ribonuclease B differs in that the mannose residue is linked $\beta 1,3$ (32), and thyroglobulin is reported to have the mannose residue linked $\alpha 1,4$ (33). This type of core unit is not unique to oligosaccharide chains with only mannose and *N*-acetylglucosamine residues, but is also found in glycopeptides which contain, in addition to mannose and *N*-acetylglucosamine, sialic acid, fucose, and galactose, such as the ones found in IgA,¹ IgE, and IgG (13).

The core unit found in the C-1 glycopeptide of IgE is quite different from the typical core just described in that there is no chitobiose unit but rather an alternating pattern of mannose and *N*-acetylglucosamine residues. In addition, the outer *N*-acetylglucosamine is linked to the underlying mannose residue by an α linkage rather than a β linkage (Fig. 4). This is, to our knowledge, the first example of an α -linked *N*-acetylglucosamine in a circulating glycoprotein. This core structure also differs from the core of the mannose-*N*-acetylglucosamine glycopeptide of IgM which has the following structure (7):



In this instance both mannose residues are external to the *N*-acetylglucosamine residues and are presumed to be linked β since they could not be removed with α -mannosidase.

Another feature common to several of the mannose-*N*-acetylglucosamine glycopeptides is a rather pronounced microheterogeneity; in other words, there is a common core to which varying numbers of additional mannose and, in the case of ovalbumin (34), *N*-acetylglucosamine residues are added. This form of peripheral heterogeneity is very striking in ovalbumin (34) and thyroglobulin (33) and is present to a lesser extent in the IgM glycopeptide (7). In the case of IgE there is no evidence for microheterogeneity. Data from enzymatic degradation, methylation, periodate oxidation, and total composition are all in very good agreement and show no significant number of partial residues in the C-1 glycopeptide. In contrast, the mannose-*N*-acetylglucosamine glycopeptide of IgM seems to be composed of three species of the glycopeptide present as 23, 32, and 45% of the total, containing 5, 6, and 7 mannose residues, respectively (7). It is of interest to note that in the case of IgM there are 2 of the mannose-*N*-acetylglucosamine units per heavy chain, and in the case of thyroglobulin there are 7 to 8 of the mannose-

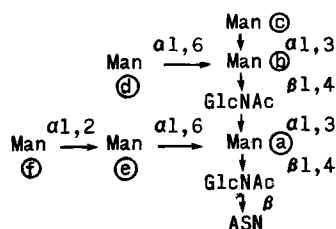


Fig. 6. Proposed structure for C-1 glycopeptide of IgE.

N-acetylglucosamine units in the human protein and 5 to 6 in the calf protein (33). In the case of the IgE C-1 glycopeptide there is only 1 unit per heavy chain. This suggests that possibly the location of a carbohydrate unit on the polypeptide chain influences the degree to which the carbohydrate unit is completed. Thus, there may be local environmental factors in the region of the growing oligosaccharide chains which affect the activities of the glycosylating enzymes. Microheterogeneity could arise in other ways as well. Variations in the core structures of the different glycopeptides could influence the activities of the glycosyltransferases which add sugar residues to the outer branches of the oligosaccharide units. In fact, with different core structures there may be different glycosyltransferases involved in the biosynthesis of the outer branches of the oligosaccharide units. Microheterogeneity could also result from degradation of completed carbohydrate units, particularly in those proteins which circulate in plasma.

In regard to our procedure for determining the sequence of such a glycopeptide, we have found that performing a methylation analysis as early as possible in the study is very helpful, since this allows one to predict the results of periodate degradations and plan what products of the periodate degradation might require separation and isolation. We have also found that sequential enzymatic degradations followed by periodate oxidation or methylation are often much more effective than sequential Smith degradations due to the difficulty of obtaining complete release of reduced fragments in the latter procedure, especially when multiple substitutions are present.

The following paper deals with the structure of glycopeptides B-1 through B-4.

Addendum—Since this manuscript was submitted for publication, Tarentino *et al.* (35) have reported that the mannose residue in the core of the type A unit of thyroglobulin is linked β rather than α as previously reported (33).

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