The Structure of the Glycopeptides of a Human γM-Immunoglobulin*

SCOT HICKMAN, ROSALIND KORNFELD,† C. KIRK OSTERLAND, AND STUART KORNFELD§

From the Departments of Internal Medicine, Biochemistry, and Preventive Medicine, Washington University School of Medicine, St. Louis, Missouri 63110

SUMMARY

Seven glycopeptide fractions, representing five different glycopeptides, were isolated from a γM-immunoglobulin obtained from a patient with Waldenström's macroglobulinemia. Two of the glycopeptides contained only mannose and N-acetylglucosamine residues in the molar ratio of 7:2, while the other three glycopeptides contained sialic acid, galactose, mannose, N-acetylglucosamine, and fucose in the molar ratio of 1:2:3 to 4:4:4:5:0.5. The structure of the oligosaccharide portion of four of the glycopeptides was investigated as follows. Sequential enzymatic degradation of the oligosaccharide chains with purified glycosidases helped to determine the sequences of the sugars. The linkages between the sugars were established by serial periodate oxidation and by methylation of the glycopeptides, followed by identification of the alditol acetate derivatives of the methylation products. Gas chromatography and mass spectrometry were used to identify the sugars present. The periodate oxidation was carried out at 60°C for 24 hours with a further addition of 1 μg pronase per milligram of material. The products were chromatographed on paper and compared to standards. The oligosaccharide portion of four of the glycopeptides was investigated as follows.

In a previous study we have described the structure of the carbohydrate moiety of human γG-immunoglobulin (1). In contrast to the majority of γG-immunoglobulins which contain only mannose and N-acetylglucosamine residues, which is distributed among four to six glycopeptides per heavy chain (2-8), although the composition of these glycopeptides has been determined in a few instances, there is limited information about the structures of the oligosaccharides. Since the γG and γM immunoglobulins share many common structural features, we decided to study the structure of the carbohydrate moieties of γM-immunoglobulin to determine what similarities they have, if any, to the γG-glycopeptide.

EXPERIMENTAL PROCEDURE

Preparation of γM-Immunoglobulin—Serum obtained from a patient with Waldenström macroglobulinemia was dialyzed against 0.0015 M phosphate buffer, pH 5.0, for 24 hours at 4°C. The precipitated euglobulin was collected by centrifugation, dissolved in a minimal volume of 0.15 M NaCl, and applied to a Sephadex G-200 column (5 x 80 cm). Elution was carried out with 0.15 M NaCl and fractions were monitored for their absorption at 280 nm. The fractions containing the γM-immunoglobulin were pooled, dialyzed against water, and lyophilized. The protein preparation was greater than 95% pure as judged by immunoelectrophoresis and zone electrophoresis on cellulose acetate membranes with the use of a Beckman microzone electrophoresis apparatus.

Preparation of Glycopeptides—In the preparation of the γM-glycopeptides we have followed the procedure described by Spragg and Clapham (4). The protein (925 mg) was incubated with 1% (w/w) pronase in 0.001 M CaCl₂. Incubation was carried out at 60°C for 24 hours with a further addition of 1% pronase at 12 hours. The pH of the reaction mixture was maintained at 8.0 with additions of NaOH. At the completion of the digestion period the solution was centrifuged and the supernatant fluid applied to a Sephadex G-25 column (3.5 x 80 cm). Elution was carried out with water and the glycopeptide material was identified by analyzing each fraction for hexose by the phenol-H₂SO₄ method (9) scaled down to one-fifth volume. The glycopeptide material, which was eluted as a single peak, was pooled, lyophilized, and subjected to a second digestion with pronase under the conditions described above. This second digest was again subjected to gel filtration on Sephadex G-25. The fractions containing the glycopeptide material were pooled, lyophilized, and applied to a Sephadex G-100 column (3 x 75 cm). A small amount of hexose-containing material was eluted with the void volume, whereas the vast majority of hexose-containing material was retarded on the column. The fractions comprising this second peak were pooled, lyophilized, and then subjected to preparative descending paper chromatography in 1-butanol-acetic acid-water (12:3:5) for 48 hours. In this solvent system, the glycopeptides remain at the origin, whereas other peptide ma-
terial migrates down the paper (4). The glycopeptides were then eluted from the origin of the paper.

Separation of Glycopeptides—Individual glycopeptides were isolated by preparative high voltage paper electrophoresis on Whatman No. 3MM paper. The glycopeptides were initially subjected to electrophoresis at 2000 volts in pyridine-acetic acid-water (1:10:289), pH 3.7, for 2 hours. This procedure resolved the glycopeptide mixture into five components (Fig. 1). Each of the glycopeptides was eluted from the paper and subjected to electrophoresis at 3000 volts in 1.24 M pyridine-acetate buffer, pH 6.4, for 1 hour, 40 min to 5 hours. Two of the glycopeptides resolved into two components (Fig. 2), giving a total recovery of seven glycopeptides.

Analytical Methods—The methods for the analysis of the carbohydrate and amino acid composition of the glycopeptides, as well as the technique for the sequential enzymatic degradation of the glycopeptides, have been described in detail in our previous publication (1).

Molecular Weight Determinations—Estimates of the molecular weight of various glycopeptides were obtained by gel filtration on Sephadex G-50 as described by Blattli and Clamp (10).

Methylation Analysis—Glycopeptides were methylated by the method of Hakomori (11) as described by Björndal and Lundblad

![FIG. 1. Initial separation of glycopeptides by high voltage electrophoresis.](image)

![FIG. 2. Second high voltage electrophoresis of the glycopeptides.](image)

### Table I

Carbohydrate and amino acid composition of γM-glycopeptides

| Glycopeptide | Yield of glycopeptide (mol/mole monomer) | Percentage of expected | Residues | Residues |
|--------------|-----------------------------------------|------------------------|----------|
|              | (mol/mole 180,000 monomer)              |                        | Gal | GlcNAc | Gal | GlcNAc | Man | Asn | Asp | Ser | Thr | Lys | Gln | Ala | His | Pro |
| I            | 0.51                                    | 25                     | 6.4  | 2.0  | 0   | 0   | 0   | 1.1 | 0.6 | 0.1 | 0.7 | 0.2 | 1.0 | 0.7 | 0.7 |
| II           | 0.18                                    | 9                      | 6.9  | 2.0  | 0   | 0   | 0   | 1.2 | 2.0 | 0.2 | 0.2 | 0.6 | 1.1 | 0.7 | 0.7 |
| III          | 1.60                                    | 79                     | 5.2  | 2.0  | 0.31| 0   | 0   | 1.1 | 1.1 | 0.3 | 0.6 | 0.3 | 0.3 | 0.2 | 0  |
| IV           | 0.75                                    | 37                     | 3    | 3.7  | 1.9 | 1.0 | 0.4 | 1.3 | 0.7 | 0.4 | 0   | 0.2 | 0.3 | 0   | 0  |
| V            | 0.39                                    | 20                     | 4    | 4    | 1.9 | 1.2 | 0.5 | 3.4 | 1.4 | 0.1 | 0.2 | 0.1 | 0.1 | 0   | 0  |
| VI           | 0.61                                    | 31                     | 3    | 4.5  | 1.9 | 1.3 | 0.5 | 1.1 | 0.7 | 0.2 | 0   | 1.0 | 0.7 | 0   | 0  |
| VII          | 0.18                                    | 9                      | 3    | 3.6  | 2.1 | 1.3 | 0.3 | 1.3 | 0.7 | 0.2 | 1.0 | 0.5 | 0   | 0   | 0  |

* Calculated on basis of 2 GlcNAc residues per mole of glycopeptides I to III and 3 or 4 mannose residues per mole of glycopeptides IV to VII.

Assuming 2 glycopeptide units of each type (I to VII) per monomer of 180,000 molecular weight.
The methods for the preparation, separation, and identification of the alditol acetate derivatives of the methylated neutral sugars by gas liquid chromatography and mass spectrometry have been previously described (1).

Serial Periodate Oxidation—Smith degradation of the glycopeptides was performed as previously described (1).

Enzymes—Neuraminidase (EC 3.2.1.18) from Vibrio cholerae and pronase were purchased from Calbiochem. β-Galactosidase (EC 3.2.1.23), β-N-acetylglucosaminidase (EC 3.2.1.59), and α-mannosidase (EC 3.2.1.24) from jack bean meal were prepared and assayed as previously described (13). A unit of activity in each case is defined as the amount of enzyme which liberates 1.0 μmole of α- or β-nitrophenol in 60 min from the appropriate nitrophenylglycoside.

RESULTS

Composition of Glycopeptides of γM-Immunoglobulin—The compositions of the seven glycopeptides obtained from the γM-immunoglobulin are shown in Table I. The data are presented as residues of sugar per oligosaccharide chain with the N-acetylglucosamine residues being set at 2 for glycopeptides I to III and the mannose values being set at 3 or 4 residues for glycopeptides IV to VII. The glycopeptides fall into two groups:

- One group (I to II) contains only mannose and N-acetylglucosamine residues, while the other group (IV to VII) contains mannose, N-acetylglucosaminine, galactose, fucose, and sialic acid residues. The glycopeptide III fraction most likely is composed of a mixture of the two types of glycopeptides, with about 80% of the molecules containing only mannose and N-acetylglucosamine. All the glycopeptides except glycopeptide V contain a single aspartic acid residue consistent with the proposal that each glycopeptide has a single oligosaccharide chain with the number of residues shown. Since the carbohydrate and amino acid composition of glycopeptides VI and VII is virtually

![Figure 3](http://www.jbc.org/)

**TABLE II**

<table>
<thead>
<tr>
<th>Glycopeptide</th>
<th>Calculated mol wt</th>
<th>Estimated mol wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1960</td>
<td>1710</td>
</tr>
<tr>
<td>IV</td>
<td>2275</td>
<td>2140</td>
</tr>
<tr>
<td>V</td>
<td>2667</td>
<td>2200</td>
</tr>
<tr>
<td>VI</td>
<td>2470</td>
<td>2275</td>
</tr>
<tr>
<td>VII</td>
<td>2475</td>
<td>2160</td>
</tr>
</tbody>
</table>

![Figure 4](http://www.jbc.org/)

one group (I to II) contains only mannose and N-acetylglucosamine residues, while the other group (IV to VII) contains mannose, N-acetylglucosaminine, galactose, fucose, and sialic acid residues. The glycopeptide III fraction most likely is composed of a mixture of the two types of glycopeptides, with about 80% of the molecules containing only mannose and N-acetylglucosamine. All the glycopeptides except glycopeptide V contain a single aspartic acid residue consistent with the proposal that each glycopeptide has a single oligosaccharide chain with the number of residues shown. Since the carbohydrate and amino acid composition of glycopeptides VI and VII is virtually
identical, it seems likely that they are derived from the same glycopeptide on the original γM-immunoglobulin. None of the sialic acid- and fucose-containing glycopeptides (IV to VII) have a full component of these sugars, indicating that there is microheterogeneity in the oligosaccharide moieties of γM-glycopeptides. There is also microheterogeneity of the mannose residues in glycopeptide I, as will be discussed below.

The relatively low yields of the various glycopeptides are due not only to the many steps involved in their isolation but also to the deliberate attempt to obtain pure glycopeptides at the expense of quantitative recovery.

Molecular Weight Determinations—The molecular weights of the various glycopeptides were estimated by exclusion chromatography (Fig. 3). As shown in Table II, the values obtained from these studies agreed reasonably well with the calculated molecular weights, although the estimated values tended to be somewhat lower than the calculated values.

Structural Studies on Glycopeptide I—The most extensive structural studies have been performed on glycopeptides I, IV, V, and VI.

### Table III

<table>
<thead>
<tr>
<th>Glycopeptide</th>
<th>Fucose</th>
<th>Galactose</th>
<th>Mannose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycopeptide I</td>
<td>2,3,4-trimethyl</td>
<td>2,3,4,6-tetraethyl</td>
<td>2,3,4,6-trimethyl</td>
</tr>
<tr>
<td>Glycopeptide IV</td>
<td>2,3,4,6-diethyl</td>
<td>2,3,4,6-trimethyl</td>
<td>2,3,4,6-diethyl</td>
</tr>
<tr>
<td>Glycopeptide V</td>
<td>2,3,4,6-diethyl</td>
<td>2,3,4,6-trimethyl</td>
<td>2,3,4,6-diethyl</td>
</tr>
<tr>
<td>Glycopeptide VI</td>
<td>2,3,4,6-diethyl</td>
<td>2,3,4,6-trimethyl</td>
<td>2,3,4,6-diethyl</td>
</tr>
</tbody>
</table>

**Fig. 5.** Proposed structure of glycopeptide I. The glycopeptide I fraction is represented as being composed of three components which differ from each other in their mannose content. Also shown are the expected methylated derivatives assuming that 23% of the molecules have the A structure, 32% have the B structure, and 45% have the C structure. As discussed in the text, mannose residues 1 and 2 could equally well be linked to mannose residue 3.
Treatment of glycopeptide I with α-mannosidase released approximately 4.4 residues of mannose out of a total of 6.4. β-N-Acetylglucosaminidase treatment, either before or after α-mannosidase treatment, failed to release significant amounts of N-acetylglucosamine. In order to gain more information about the arrangement of the mannose residues in glycopeptide I, the glycopeptide was methylated, and the alditol acetate derivatives of the methylated mannose residues were separated by gas chromatography, as shown in Fig. 4. Estimation of the areas under the peaks revealed that there were approximately 3.5 residues of 2,3,4,6-tetramethylmannose, 1.1 residues of 3,4,6-trimethylmannose, 0.32 residue of 2,3,4-trimethylmannose, and 1.4 residues of 2,4-dimethylmannose (Table III). The finding of partial residues of several of the methylated derivatives suggests that there is microheterogeneity of the mannose residues in the glycopeptide. When the glycopeptide was treated with α-mannosidase to remove the susceptible mannose residues and the residual glycopeptide was methylated, the only methylated mannose derivative found was 2,3,4,6-tetramethylmannose.

These data are compatible with the structures shown in Fig. 5. According to this proposal the basic structure (A) contains 5 mannose residues. In addition, since the methylation data indicate that glycopeptide I has microheterogeneity in the mannose residues, it must also contain structures with 6 and 7 mannose residues, as shown in Fig. 5, B and C. On the basis of the methylation data, we have calculated that approximately 23% of the glycopeptide I molecules have Structure A, 32% have Structure B, and 45% have Structure C. With this distribution of molecules one would expect to find an average of 6.4 mannose residues for each 2 N-acetylglucosamine residues, which was the observed value (Table I). According to these structures, α-mannosidase would release all of the mannose residues except Residues 0 and 4 which would give tetramethyl derivatives upon methylation. Neither of the N-acetylglucosamine residues would be susceptible to β-N-acetylglucosaminidase since they are internal to mannose residues 0 and 4. This series of structures does not uniquely account for all the data. As long as the proper number of mannose residues and the proper number of each type of linkage is preserved, the structures shown can be rearranged in other patterns. For example, mannose residues 0 and 5 could equally well be linked to mannose residue 3.

Further information about the structure of glycopeptide I was obtained from serial periodate oxidation (Table IV). The first periodate oxidation destroyed 5 mannose residues, as predicted from the methylation data. Following the second periodate oxidation, 62% of the remaining mannose was destroyed, as well as 1 of the 2 N-acetylglucosamine residues. These data are consistent with the following possible arrangements of the core sugars.

\[
\text{Man}^{(4)} \xrightarrow{1 \rightarrow 3 	ext{ or } 4} \text{GlcNAc} \xrightarrow{2,4} \text{GlcNAc} \rightarrow \text{Asn}
\]

or

\[
\text{Man}^{(3)} \xrightarrow{1 \rightarrow 3, 4, 	ext{ or } 6} \text{GlcNAc} \xrightarrow{2,4} \text{GlcNAc} \rightarrow \text{Asn}
\]

The fact that 1 of the N-acetylglucosamine residues was destroyed on the second periodate oxidation indicates that mannose residue 4 must have been destroyed during the first periodate oxidation. Thus, it seems likely that mannose residue 3 is unsubstituted.

**Structural Studies on Glycopeptides IV, V, and VI**—The results of a series of enzymatic degradation studies performed on glycopeptides IV, V, and VI are summarized in Table V. These data indicate that the oligosaccharide chain of each of these glycopeptides is a branched structure with two nonreducing termini, one with the sequence galactose → N-acetylgalactosamine → mannose and the other with the sequence sialic acid → galactose → N-acetylgalactosamine → mannose. About one-third of the oligosaccharide chains of glycopeptides V and VI seem to have 2 terminal sialic acid residues. Glycopeptide V has, in addition, 1 terminal mannose residue since α-mannosidase treatment of the native glycopeptide released a significant amount of mannose and the methylation studies (Table III) revealed 1 residue of tetramethylmannose.
FIG. 6. Proposed structures of the γM-immunoglobulin glycopeptides. Note that the sialic acid residues in glycopeptides IV and V could equally well be linked to either galactose residue.

After removal of the sugars in the two branches, the remaining cores in each case contained 1 mannose, 2 N-acetylglucosamine, and somewhat less than 1 fucose residues.

Methylation of the intact glycopeptides IV, V, and VI revealed that each contained approximately 1 residue of 2,4-dimethylmannose in addition to 2 residues of 3,4,6-trimethylmannose (Fig. 4 and Table III). Further, each of the glycopeptides had slightly less than 1 residue of 2,3,4,6-tetramethylgalactose and approximately 1 residue of 2,3,4-trimethylgalactose. These findings are compatible with the results of the sequential enzymatic degradation studies. In addition, they indicate that the sialic acid is linked α2 → 6 to the galactose residue in all cases and that the N-acetylglucosamine residues in the outer branches are linked β1 → 2 to the underlying mannose residues.

Periodate oxidation of glycopeptide IV resulted in the destruction of all the sugar residues except for 1 mannose and 2 N-acetylglucosamine residues (Table IV). The loss of the sialic acid, fucose, galactose, and 2 outer chain mannose residues would be predicted. Since the outer chain N-acetylglucosamine residues were also destroyed, the Gal → GlcNAc linkage must be β1 → 6. With the second round of periodate oxidation 1 of the 2 N-acetylglucosamine residues in the core was destroyed as well as 40% of the remaining mannose residue. Because of the shortage of material, serial periodate oxidation could not be performed on the other glycopeptides.

On the basis of these data, the structures of glycopeptides IV, V, and VI are proposed as shown in Fig. 6.

DISCUSSION

The γM immunoglobulin used in the present study contains oligosaccharide units of two basic types, one of which contains only mannose and N-acetylglucosamine residues (type B chains of Johnson and Clamp (8)), whereas the other contains sialic acid, galactose, mannose, N-acetylglucosamine, and fucose (type A chains of Johnson and Clamp). Similar findings have been reported by others (2-8). Among different γM-immunoglobulins, the oligosaccharide units containing only mannose and N-acetylglucosamine residues display significant variation in composition, containing between 2 to 8 mannose residues and 2 to 3 N-acetylglucosamine residues per unit (2-4, 6-8). At least part of this variation can be explained on the basis of different degrees of microheterogeneity of the oligosaccharide units, as illustrated by glycopeptide I.

Although the low yield of glycopeptides combined with micro-
The contribution by these contaminants, as reflected in the con-
previously mentioned, glycopeptide III is contaminated with
residues in type CA was therefore obtained by adding the residues
of each sugar from IV, V, and VI. The two glycopeptides I (II)
and III each contain an oligosaccharide of the CB type. As
previously mentioned, glycopeptide III is contaminated with
minor amounts of the RA type of glycopeptides that lacks sialic
acid and hence did not migrate in the electrophoretic separation.
The contribution by these contaminants, as reflected in the con-
tent of galactose, has been subtracted here, leaving the composi-
tion of glycopeptide III as 6.9 residues of mannose and 2.0 resi-
dues of N-acetylgalactosamine. The total number of residues in
type B was therefore obtained by adding the residues of each
sugar from I and III.

<table>
<thead>
<tr>
<th>Total residues per monomer</th>
<th>CA type</th>
<th>CB type</th>
<th>Predicted</th>
<th>Observed in intact protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of units per 180,000 mol wt monomer</td>
<td>6</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of residues</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fucose</td>
<td>3</td>
<td>0</td>
<td>3.2</td>
<td>9.2</td>
</tr>
<tr>
<td>Mannose</td>
<td>20</td>
<td>26.6</td>
<td>46.6</td>
<td>48.4</td>
</tr>
<tr>
<td>Galactose</td>
<td>12</td>
<td>0</td>
<td>12.4</td>
<td></td>
</tr>
<tr>
<td>N-Acetylgalactosamine</td>
<td>24</td>
<td>8</td>
<td>32</td>
<td>41.6</td>
</tr>
<tr>
<td>Sialic acid</td>
<td>7.5</td>
<td>0</td>
<td>7.5</td>
<td>7.2</td>
</tr>
</tbody>
</table>

a Terminology of Johnson and Clamp (8).
b See Reference 3.

heterogeneity of structure makes it difficult to determine with
certainty the number of oligosaccharide units per γM molecule,
or results suggest that there are 10 oligosaccharide units per
monomer of 180,000 molecular weight, with 6 units of the type
CA chains and 4 units of the type CB chains (Table VI). This
number of oligosaccharide chains is consistent with previous
reports which have shown that γM-monomers from different
patients with Waldenström's macroglobulinemia contain between
8 and 12 oligosaccharide units (2–8). Recently, Shimizu et al.
(14) have shown that the five oligosaccharide chains of two
different γM-immunoglobulins have specific locations on the
constant portion of the heavy chain. One of the oligosaccharide
chains was shown to be located in the Fd fragment of the heavy
chain at approximately position 170, another in the hinge region
near position 230, and the other three in the Fc region (14). The
localization was made possible by the finding that each of the five
glycopeptides had a unique amino acid composition. Using the
data of Shimizu et al. (14), we can tentatively place glycopeptide
V in the Fd fragment, glycopeptide VI (and VII) in the hinge
region, and the other glycopeptides in the Fc region.

While the five glycopeptides have different locations along the
heavy chain, as well as different compositions, all of their struc-
tures appear to be derived from a common core, consisting of

\[
\text{Man}^4 \rightarrow \text{Man}^3 \rightarrow [\text{GlcNAc} \rightarrow \text{GlcNAc} \rightarrow \text{Asn}]
\]

As can be seen in Fig. 5, glycopeptide I consists of the common
core plus 2 to 4 additional mannose residues. Presumably
glycopeptides II and III have structures similar to glycopeptide
I. Glycopeptides IV and V (Fig. 6) contain the common core
with two outer branches consisting of sialic acid → Gal → GlcNAc
and Gal → GlcNAc sequences linked to mannose residues 10
and 11. In addition, there is a fucose residue linked to 1 of the
2 GlcNAc residues in the core. Glycopeptide V differs from
glycopeptides IV and VI in that it contains a 4th mannose residue
which is linked to 1 of the GlcNAc residues in the core. This
extra mannose residue in glycopeptide V appears to be different
from mannose 1 in glycopeptide I in that the former can be
released by α-mannosidase and the latter cannot be released.

The structure proposed for glycopeptide I differs significantly
from the structure which Johnson and Clamp (8) have recently
proposed for a γM-immunoglobulin glycopeptide which contains
7 mannose and 2 N-acetylgalactosamine residues. On the basis of
periodate oxidation and enzyme degradation studies, these
authors suggested that the glycopeptide was an unbranched
chain with the following structure:

\[
\text{Man}^1 \rightarrow 2 \rightarrow 4 \rightarrow \text{Man}^1 \rightarrow 2 \rightarrow 4 \rightarrow \text{Man}^1 \rightarrow 2 \rightarrow 4
\]

\[
\text{Man}^1 \rightarrow 2 \rightarrow 4 \rightarrow \text{Man}^1 \rightarrow 3 \rightarrow 4 \rightarrow \text{GlcNAc} \rightarrow 1 \rightarrow 3 \rightarrow \text{Man}^1 \rightarrow 3
\]

Such a structure is incompatible with our data on glycopeptide I.
The explanation for this discrepancy may be that the glycopep-
tides derived from different γM-immunoglobulins have different
structures. Since our studies have been confined to a single
γM-immunoglobulin, we cannot resolve this point.

The factors that determine the biosynthesis of these different
carbohydrate units have yet to be elucidated. Since each of the
glycopeptides has a fixed position on the constant portion of the
γM heavy chain, it seems likely that the amino acid sequence in
the region of the oligosaccharide significantly influences the
activities of at least some of the various glycosyltransferases
involved in the biosynthesis of the oligosaccharide chains. This
could result in the synthesis of oligosaccharides with different
structures. Several investigators (15–18) have proposed
that the triplet sequence Asn-X-(Ser or Thr) may be the recogni-
tion signal for the enzyme which attaches the initial N-acetyl-
glucosamine to the asparagine residue of the peptide backbone,
thus initiating the oligosaccharide unit. If the other glycosyl-
transferases were influenced by the amino acid sequences or the
polypeptide conformation in the region of the growing oligosac-
charide chain, the result could be the production of a variety of
oligosaccharides with different, but related, structures. The
finding that several light chains are devoid of carbohydrate units
in spite of having a suitable triplet sequence suggests that the
conformation of the polypeptide may indeed influence the ac-
tivity of the glycosyltransferases (14, 17). In addition, vari-
ations in the core structures of the different glycopeptides could
influence the action of the glycosyltransferases which add the
sugar to the outer branches of the oligosaccharide units. This
variation could be manifest either as differences in sugar sequences
or differences in linkages between sugars (or both). To date we
have only limited information about the core structures of the
five glycopeptides. The results of the serial periodate oxidation
of glycopeptides I and IV are compatible with three different core structures (Table IV). Similar studies have not been done with the other glycopeptides.

The structures of the γM-glycopeptides, especially glycopeptides IV to VII, have a marked resemblance to the structure of the γG-glycopeptides (1). Similarities are exhibited both in the sequences of the sugars and in the linkages between the sugars. The sugar sequences in glycopeptides IV and VII seem to be identical with the sugar sequences in one of the γG-glycopeptides. The glycopeptides of both types of immunoglobulins contain in their outer branches sialic acid linked α2 → 6 to galactose and N-acetylglucosamine linked β1 → 2 to mannose. The linkages of the mannose residues in the core are identical in both instances. The reason why γM-immunoglobulin contains more carbohydrate units than γG-immunoglobulin is still a mystery.

Recently Yamaguchi et al. (19) have reported the complete sequence of a glycopeptide obtained from TAKA-amylase A. The sugar sequence and the linkages between the sugars bear a remarkable similarity to those found in glycopeptide I.

REFERENCES
The Structure of the Glycopeptides of a Human γM-Immunoglobulin
Scot Hickman, Rosalind Kornfeld, C. Kirk Osterland and Stuart Kornfeld


Access the most updated version of this article at http://www.jbc.org/content/247/7/2156

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/247/7/2156.full.html#ref-list-1