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AMINO ACID SEQUENCE AND LOCATION OF THE THREE GLYCOPEPTIDES IN THE Fc REGION OF HUMAN IMMUNOGLOBULIN D

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SUMMARY

Amino acid sequence and the location of three glycopeptides of the Fc fragment obtained by limited proteolysis with trypsin of an intact myeloma protein(NIG-65) has been determined. These glycopeptides have glucosamine oligosaccharides(designated GlcN) which are attached to Asn-68, Asn-159, and Asn-210 by N-glycosidic linkages. Of these, Glcn-159 is characteristic of the δ chain and has no counterpart position in any of the other classes. On the other hand, GlcN-68 is shared by γ, μ, and ε chains, and GlcN-210 by α and μ chains, respectively. Although different classes of human immunoglobulins differ in the number and the kinds of oligosaccharides, the sites are often homologous and are related to the basic immunoglobulin domain structure.

Although the five principal classes of human immunoglobulins differ considerably in the content and the kinds of carbohydrates, they often occupy the homologous positions along the heavy chains. The γ1 chain of human IgG1 has a single oligosaccharide and it is located in the Fc region(1), the μ chain of human IgM has three(2) and the ε chain has two oligosaccharides in the Fc region(3). The similar data have recently been reported for the α1 chain of human IgA1(4). In all these cases the oligosaccharide contains glucosamine, which is covalently bonded to aspargine by an N-glycosidic linkage. Although the data of the kinds and locations of oligosaccharide are now available four of the five principal classes of human immunoglobulins, the fifth class the δ chain is not yet available. It is necessary to have such data for the δ chain before having a general view on the relationship between immunoglobulin classes and their biological roles and functions. In this communication we will report the amino acid sequence and the location of three glycopeptides isolated from the Fc region of the δ chain of human IgD.

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MATERIALS AND METHODS

Unless otherwise mentioned all the procedures of the experiment were done at 4°C. IgD was purified from the plasma of a patient with multiple myeloma in the following ways. Approximately 300 ml of the frozen plasma was taken in 200 ml of 0.15 M NaCl-0.12% NaN_3(pH 7.2) containing 25 mM ε - amino caproic acid and 10 mM $CaCl_2$. Saturated $(NH_4)_2SO_4$ solution was added , and the precipitates obtained between 1.5 and 1.8 M $(NH_4)_2SO_4$ concentration was collected. The precipitate was dissolved in 10 mM phosphate buffer-0.05% NaN_3 (pH 8.0) containing 10 mM ε - amino caproic acid and dialysed against the same buffer. The solution was applied to a DEAE-Sephadex A-25 column, and most of the IgD was eluted with the same buffer containing 80 mM NaCl. The IgD pool was purified further by gel filtration with a Bio-Gel A-5m column in 0.1 M Tris·HCl-0.15 M NaCl-0.05% NaN_3(pH 8.0) containing 10 mM ε -amino caproic acid. Fractions were assayed by immunodiffusion and immunoelectrophoresis for a variey of plasma proteins. The purity of IgD was also tested by polyacrylamide gel electrophoresis before and after the treatment with SDS and also by N- and C-terminal analyses.

Purified IgD(600 mg) dissolved in 30 ml of 10 mM phosphate buffer contaning 0.05% NaN_3(pH 8.0) was digested with 6 mg of trypsin(TPCK-treated, 2 x crystallised, Worthington) at 37°C for 2 min. The reaction was halted by adding 12 mg of lima bean trypsin inhibitor(2 x crystallised, Worthington). Following the removal of the precipitate by centrifugation at 28,000 x g for 30 min, the clear supernatant was fractionated into the Fab and the Fc fragments by column chromatography with DEAE-Sepharose CL-6B(5). For reduction and aminoethylation the purified Fc fragment(150 mg) was dissolved in 6.75 ml of 0.2 M Tris HCl-5 mM EDTA buffer, pH 8.6 containing 6.8 M guanidine HCl, and treated as described(6).

Aminoethylated Fc fragment(120 mg) dissolved in 15 ml of 50 mM NH_4HCO_3, pH 7.8 was digested with 5 mg of S. aureuse V8 protease(lyophilised, Miles) at 37°C for 24 h, and the digest was lyophilised. The glycopeptides were separated on a column of DEAE-Sephadex A-25(1.5 x 42 cm) equillibrated with 10 mM NH_4HCO_3-10% 1-propanol. Peptides were eluted at a flow rate of 20 ml/h with a linear gradient of increasing NH_4HCO_3 concentration from 0.01-0.6 M. Further purification of the peptides was performed either by gel filtration on a column of Bio-Gel P-6(1.5 x 98 cm) using 0.1 M NH_4HCO_3, or by a column of CM-Sephadex C-25(0.9 x 40 cm) as described(ms submitted).

The methods for amino acid analysis, for digestion with carboxypeptidases A and P, and for sequence determination by manual Edman degradation have been described(6,7). Oligosaccharide-containing peptides are numbered and located according to the order of their occurences in the sequence(ms in preparation).

RESULTS AND DISCUSSION

When the purified IgD protein was tested serologically with specific antisera at concentration of 0.5-10.0 mg/ml, it was found to react only with anti-δ and anti-μ antisera. The molecular weight of the protein was estimated to be in the range between 168,000 and 172,000 with a mean of 170,000. It contained 12.3% carbohydrates and virtually all of them including sialic acid, neutral sugars, glucosamine, and galactosamine were recovered from the δ heavy chain. Among the two kinds of hexosamines, whereas galactosamine was found only in the Fd fragment, all glucosamine moieties were recovered from the Fc fragment.

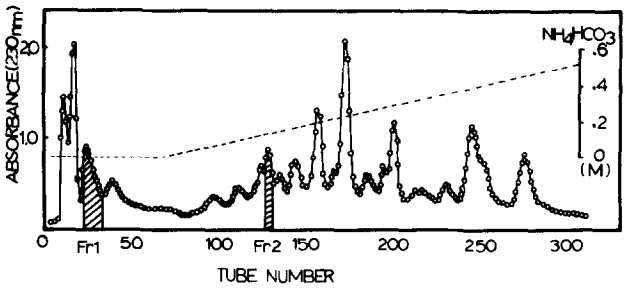

Fig. 1 Separation of S. aureus V8 protease digest of completely reduced and aminoethylated Fc fragment of IgD. The digest(ca. 120 mg) was applied to a column(1.5 x 42 cm) of DEAE-Sephadex A-25 equilibrated with 10 mM NH_4HCO_3-10% 1-propanol, pH 8.0. Peptides were eluted at a flow rate of 20 ml/h with a linear gradient of increasing NH_4HCO_3 concentration from 0.01-0.6 M, and purified further as described in the text. Frl and Fr2 indicate the peptide fractions from which three glucosamine-containing peptides are isolated.

Following the chromatography on a column of DEAE-Sephadex A-25 of S. aureuse protease digestion of the completely reduced and aminoethylated Fc fragment, an elution profile shown in Fig. 1 was obtained. In this chromatogram two fractions designated Frl and Fr2 were found to have glucosamine oligosaccharide following the amino sugar analysis. From the Frl two glucosamine oligosaccharide-containing peptides, GlcN-1 and GlcN-2 were isolated by gel filtration with Bio-Gel P-6 and ion exchange chromatography with CM-Sephadex C-25(not shown). Another glucosamine oligosaccharide-containing peptide, GlcN-3 was recovered from the Fr2 and was purified further by gel filtration as described. The amino acid composition and glucosamine content of these glycopeptides are shown in Table 1. GlcN-1 was a nonapeptide and was sequenced throughout by nine steps of Edman degradation. GlcN-2 was 21-peptide containing a tryptophan residue at the C-terminal. The sequence was established by twenty steps of Edman degradation and carboxypeptidase A digestion. GlcN-3 was a 13 peptide and was sequenced throughout by thirteen steps of Edman degradation. The results are summarised in Table 2. Despite no sequence homology among these glycopeptides, each has a characteristic sequence, Asn-X-Thr/Ser(where X can be any amino acid), which can serve as an acceptor site for carbohydrate addition through the dolichol phosphate N-linked pathway(8).

Table 1. Amino acid compositions of glycopeptides of Fc_{δ} fragment

	GlcN 1	GlcN 2	GlcN 3
Lysine			
Histidine	1.0		
Arginine	0.7	1.3	1.9
Aspartic acid	1.0	1.0	2.1
Threonine		2.5	1.0
Serine	2.9	2.0	2.8
Glutamic acid	2.0	1.1	1.0
Proline		4.5	
Glycine	1.0	1.7	
Alanine		2.1	1.0
Valine		1.0	
Methionine			
Isoleucine			
Leucine			2.9
Tyrosine			
Phenylalanine		1.6	
Tryptophan		0.8	
Galactosamine			
Glucosamine	1.1	3.1	3.3

When all the five principal human heavy chain classes are compared with respect to the number, the kinds, and the location of carbohydrate moieties, some of them are located at the common positions while other seems characteristic of each heavy chain(Fig. 2). In contrast to the γ1 chain, the Fcδ has three GlcN oligosaccharides. Of these, GlcN-1 is shared by the γ1 chain and also by μ and ε chains. On the other hand the GlcN-2 appears to be characteristic of the δ chain. It has no counterpart position in any of the other classes. Although the GlcN-3 is absent in the γ1 chain, it has a counterpart position in the C-terminal extra sequences of both the μ and α chains. Despite the latter two heavy chains share the same acceptor sequence, Asn-Val-Ser, in the C-terminal extra sequence, the δ chain has a different one, Asn-Ala-Ser.

Table 2. Amino acid sequence of glycopeptides of Fc_{δ} fragment

GlcN 1	Arg-His-Ser-Asn(CHO)-Gly-Ser-Gln-Ser-Gln
GlcN 2	Val-Asn(CHO)-Thr-Ser-Gly-Phe-Ala-Pro-Ala-Arg-Pro-Pro-Pro-Gln-
	Pro-Gly-Ser-Thr-Thr-Phe-Trp
GlcN 3	Asp-Ser-Arg-Thr-Leu-Leu-Asn(CHO)-Ala-Ser-Arg-Ser-Leu-Glu

This Val-Ala interchange in the triplet seems to be fortuitous, since only a little homology exist between δ and μ, and between δ and α in the C-terminal extra sequence(about 26%, in each case) while that between μ and α is about 70%. In the mouse μ chain there is also glucosamine oligosaccharide which is attached to an acceptor sequence of Asn-Val-Ser in the C-terminal extra sequence, and the sequence is homologous with the corresponding region of the human μ chain as much as 88%(9). This suggests that there are small DNA seg-

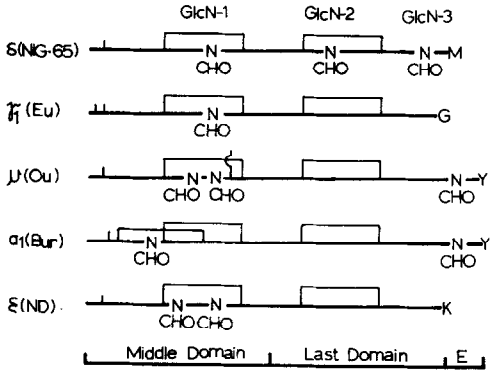

Fig. 2 Schematic diagram of location of carbohydrate moieties in human δ, γ1, μ, ε, and α1. CHO in the figure shows glucosamine-containing oligosaccharides. The second domain corresponds to Cδ2, Cγ2, Cμ3, Cε3, and Cα2, whereas the last domain to Cδ3, Cγ3, Cμ4, Cε4, and Cα3, respectively. Single letter code for amino acid is taken from Dayhoff(13).

ments which have either already been attached to the CH3 domain exon, or originally existed as discrete segments in introns. They are joined with the CH3 exon prior to expression into a functional domain-coding gene.

As to the biological functions of the carbohydrate in the immunoglobulins, some significant observations have been made in the secretion from the plasmacytes, in the skin fixation, and in the binding of complements(10). It increases solubility of immunoglobulin molecules and may facilitate placental transfer for some immunoglobulin classes(11). Because of the massive carbohydrate moieties molecules are shown to be protected from catabolic degradations (12). As shown Fig. 2, δ, μ, and α heavy chains have GlcN-oligosaccharide in the C-terminal extra sequence. It suggests their possible significance of the binding to cell membrane, either specifically or non-specifically. The fact that homologous carbohydrates are located at homologous positions in human as well as in animal immunoglobulins not only indicates that the acceptor sequence in the constant region has been converved throughout the long history of the diversity in immunoglobulins of different classes and species, but also suggest their structural and functional significance.

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