Structures of the O-glycosidically Linked Oligosaccharides of Human IgD*

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In the previous communication (Mellis, S. J., and Baenziger, J. U. (1983) J. Biol. Chem. 258, 11546–11556), the structures of the oligosaccharides present at the 3 asparagine glycosylation sites of a human IgD myeloma protein were defined. In this communication, we present the structures of the O-glycosidically linked oligosaccharides located in the hinge region of IgD:WAH. Three or four threonine residues and one serine residue in the region bear O-glycosidically linked oligosaccharides. Approximately 50% of these molecules have the structure \( \text{Gal} \beta 1\rightarrow 3 \text{GalNAc} \) which is identical with the structure of the predominant oligosaccharide in the hinge region of human IgA, myeloma proteins (Baenziger, J. U., and Kornfeld, S. (1974) J. Biol. Chem. 249, 7270–7281). The remainder of the oligosaccharides contain 1 or 2 residues of \( \text{N-acetylneuraminic acid} \) and have the structures \( \text{NeuAc} \alpha 2 \rightarrow 3 \text{Gal} \beta 1 \rightarrow 3 \text{GalNAc} \) (50%), \( \text{Gal} \beta 1 \rightarrow (\text{NeuAc} \alpha 2 \rightarrow 6) \text{GalNAc} \) (12%), and \( \text{NeuAc} \alpha 2 \rightarrow 3 \text{Gal} \beta 1 \rightarrow 3(\text{NeuAc} \alpha 2 \rightarrow 6) \text{GalNAc} \) (8%). The sialylated molecules have not been encountered previously on other human immunoglobulin heavy chains. These structures, however, have been described on a number of secreted and membrane glycoproteins. Examination of oligosaccharides isolated from different subregions of the IgD hinge indicated that a specific distribution of the sialylated structures among the glycosylated amino acids of the hinge region is not likely.

Only a small number of serum proteins are known to contain both O- and N-glycosidically linked oligosaccharides. Among these are human IgA (1, 2), human IgD (3–5), and rabbit IgG (6). In the preceding paper (7), we determined the structures of the oligosaccharides located at each of the asparagine glycosylation sites of a human IgD myeloma protein IgD:WAH. We now present data establishing the structures of the oligosaccharides O-glycosidically linked to serine and threonine residues present in the hinge region of IgD:WAH.

IgD, like IgG, and IgA, contains a segment of heavy chain between the Fd and Fc regions termed the hinge region. In the \( \delta \) and \( \alpha \), heavy chains, this region contains the only sites of O-glycosidically linked carbohydrate among all the immunoglobulin classes of humans (8). The hinge region is believed to confer flexibility to immunoglobulin molecules and may transduce conformational signals from the antibody-combining site to biological effector domains of Fe (9). Whereas the function of the secreted form of IgD is unknown, the membrane form of this immunoglobulin is believed to mediate important immunoregulatory functions in B cells (reviewed in Refs 10–12). It has been proposed that the hinge region of IgD is of special importance in these biological processes (13). Dr. Frank Putnam, who with his co-workers has determined the complete amino acid sequence of IgD:WAH (14), has provided us with glycopeptides encompassing specific portions of the IgD hinge. In this paper, we present the detailed structural characterization of the O-glycosidically linked oligosaccharides of IgD as well as information regarding the distribution of different oligosaccharide structures within the hinge region.

**EXPERIMENTAL PROCEDURES**

**Materials**

*Human Myeloma IgD:WAH-* Hinge region glycopeptides were kindly provided by Dr. Frank Putnam, Department of Biology, Indiana University, Bloomington. The complete amino acid sequence of IgD:WAH has been established (14).

**Reagents**—Triethylamine (Eastman) was redistilled once by flash evaporation. Glacial acetic acid was purchased from Fisher and boric acid from Sigma. The DA-X-8 resin was purchased from Durrum Chemical Corp. The remainder of materials used have been described in the previous communication (7).

**Enzymes**—The purification of enzymes from jack bean meal, *Clostridium perfringens*, and *Diplococcus pneumoniae* have been described previously (7). Neuraminidase from Newcastle Disease Virus was the generous gift of Dr. James Paulson, Department of Biological Chemistry, UCLA School of Medicine.

**Methods**

The methods employed have been described in the previous paper (7) with the following additions.

**Alkaline Degradation**—Fifty-nmol aliquots of hinge region glycopeptides were subjected to alkaline degradation at 37 °C in 50 μl of 0.1 M NaOH containing 0.2 or 0.4 M NaBH₄ (847.5 mCi/mmol) for 36 or 56 h, respectively. Results were similar under both conditions. The reaction mixture was acidified to pH 4.6 with 1 M HCl (H⁺ form, 200–400 mesh). Oligosaccharides were eluted with 7 ml of H₂O after which peptide was eluted in 7 ml of 0.5 M NH₄OH. Oligosaccharides were then re-N-acetylated by successive additions of 100 μl of 5% acetic anhydride in saturated NaHCO₃ three times.

Na was removed by passage over AG-50W-X12.

**Ion Suppression Amine Adsorption HPLC**—This method fraction-
ates anionic complex carbohydrates on the basis of net carbohydrate content as well as linkage positions of anionic moieties (15, 16). Chromatography was performed on a Varian Model 5000 liquid chromatograph with a MicroPak AX-5 column (4 mm × 30 cm) (Varian). Flow was 1 ml/min. The mobile phase consisted of a linear gradient produced by mixing two solvents from reservoirs A and B. Reservoir A consisted of 3% (v/v) acetic acid in water (H₂O), titrated to pH 5.5 with triethylamine (Buffer A). Reservoir B contained 3% (v/v) acetic acid in H₂O, titrated to pH 5.5 with triethylamine (Buffer B). The initial ratio of Buffer A:Buffer B was 95:5 and the proportion of Buffer B was increased at a rate of 1%/min. For analytical chromatograms, 3H-oligosaccharides were injected in up to 300 μl of starting buffer and the eluate collected directly into scintillation vials at 0.3-min intervals. Water and 3a70 complete mixture (Research Products Inc.) were added for scintillation counting. For preparative fractionation, the eluate was collected in glass tubes and aliquots were removed for scintillation counting.

Anion Exchange HPLC on MicroPak AX-10—Anionic oligosaccharides were fractionated analytically as well as preparatively on the basis of net charge as described in Ref. 17.

Borate Anion Exchange HPLC—Monosaccharides were analyzed as their NaB[3H]₄ reduced derivatives on DA-X8-11 as described by Barr and Nordén (18) except that 0.3-m1 fractions were collected.

Analytical Methods—Amino acid analysis was performed following 24-h hydrolysis in 6 N HCl using a Waters HPLC amino acid analysis system. Neutral and amino sugars were determined by gas chromatography as described previously (19). Sialic acid was measured by a modification of the thiazolidinecarboxylic acid assay of Warren and Goodson (20). Methylation—Mass Spectrometry—Individual HPLC purified oligosaccharides were permethylated by the method of Hakomori (21). For methylation analysis of neutral and amino sugars, the permethylated products were extracted in chloroform, chromatographed on silica gel (22), hydrolyzed, reduced, and acetylated as described by Stellner et al. (23). For analysis of methylated sialic acids and 2-amino-2-deoxyhexitols, methanolysis and acetylation were performed as described previously (24, 25). Permethylated hexopyranosyl-2-acetamido-2-deoxyhexitols were also examined (26). Analyses were performed on Finngan Model 2300 and 3000 gas chromatographs using a column (2 mm × 2 m) packed with 3% OV-17 on Gas Chrom Q (100/120 mesh). For analysis of hexitol and aminohexitol derivatives, the temperature was increased from 150 to 250 °C at a rate of 4 °C/min. The temperature for sialic acid derivatives was increased from 200 to 250 °C at 6 °C/min. Permethylated hexopyranosyl-2-acetamido-2-deoxyhexitols were chromatographed isothermally at 240 °C. The methane chemical ionization method described by Laine (27) was used as described previously (7). Selected ion recording of heoxose (28), amino sugar (28), and sialic acid (7) derivatives has been described. Selected ion monitoring was used for analysis of 2-amino-2-deoxyhexitols and hexopyranosyl-N-acetamido-2-deoxyhexitols. The ions selected were based upon the studies of Finne and Rauvala (25) and Mononen, et al. (26) as follows. 2-Amino-2-deoxyhexitols: 88, 117, 130, 133, 161, 174, 205, 218, 246, 262, 290, 318. Hexopyranosyl-N-acetamido-2-deoxyhexitols: 88, 130, 174, 187, 219, 278, 304, 356, 378, 381, 466, 422.

RESULTS

The complete amino acid sequence of IgD:WAH has been reported and the locations of the four or five oligosaccharides O-glycosidically linked via GalNAc to hinge region Ser/Thr residues determined (14). We determined the carbohydrate composition for a glycopeptide encompassing all of the O-glycosylation sites of the hinge region (Table I). The results indicated that Gal and GalNAc were present in nearly equimolar amounts and that sialic acid was also present at approximately 50% of this amount. As the structure of the predominant O-glycosidically linked oligosaccharide present in the hinge region of human IgA has been determined to be Galβ1--3GalNAc (1), the compositional data we obtained suggested that the same structure may be present in the hinge region of IgD:WAH with the additional presence of sialylated derivatives.

A glycopeptide encompassing all glycosylation sites of the hinge region was subjected to alkaline degradation in 0.1 N NaOH containing 0.4 m NaB[3H]₄. During the β-elimination of the oligosaccharides from the glycopeptide, tritium was introduced into the reducing termini by reduction with the NaB[3H]₄. This reaction was monitored by amino acid analysis before and after alkaline degradation and it was determined that all GalNAc residues were released from the glycopeptide concomitant with the destruction of 4 threonine residues (Table II). The expected conversion of 1 residue of serine to alanine was not observed; however, this is believed to be a technical artifact due to reasons described in a later section.

The 3H-oligosaccharides were initially analyzed by anion exchange HPLC on MicroPak AX-10 (17). The chromatographic profile obtained is displayed in Fig. 1. Three species were observed and two of these co-migrated with neutral and monosialylated complex type oligosaccharide standards, respectively. A third minor species chromatographed in a position intermediate between the elution positions of a di- and trisialylated complex type oligosaccharide. This result suggested the presence of oligosaccharides containing 0, 1, and 2 or 3 residues of sialic acid, respectively. The oligosaccharide mixture was also analyzed by ISSA-HPLC (15). This method fractionates oligosaccharides predominately on the basis of net carbohydrate content; however, the linkage position of anionic moieties significantly influences the elution positions of smaller and more highly charged oligosaccharides (15, 16). The chromatographic profile obtained from the analysis of the oligosaccharide mixture on ISSA-HPLC is shown in Fig. 2A. Five distinct species were resolved and have been designated H1, H2, H3, H4, and X.

In order to establish the relationship between the three fractions detected by anion exchange HPLC and the five oligosaccharide species identified by ISSA-HPLC, the 3H-oligosaccharides were preponderantly fractionated by anion exchange HPLC and then examined individually by ISSA-HPLC.
HPLC. The converse experiment was also performed in which oligosaccharides were preparatively fractionated by ISSA-HPLC and then individually analyzed by anion exchange HPLC. It was thereby determined that ISSA-HPLC fractions H1 and X corresponded to neutral species, H2 and H3 corresponded to species which co-eluted with monosialylated complex type oligosaccharides, and H4 was the species that eluted in a position intermediate between the elution positions of di- and trisialylated complex type oligosaccharides on anion exchange HPLC.

In order to establish that the anionic character of species H2, H3, and H4 were due to the presence of sialic acid, the total oligosaccharide mixture was digested with neuraminidase from C. perfringins and the products were analyzed by ISSA-HPLC (Fig. 2B). This digestion caused the disappearance of species H2, H3, and H4 from the chromatographic profile with a concomitant increase in the ratio of species H1 to X (Fig. 1B). Similar digestion of a mixture of ISSA-HPLC-purified H2, H3, and H4 resulted in the formation of only one species which co-migrated with H1. The results of these digestions indicated that H2, H3, and H4 were sialylated derivatives of the neutral oligosaccharide H1. The results also indicated that H2 and H3 were most probably linkage isomers of monosialylated H1 because these molecules co-migrated on ISSA-HPLC after removal of the sialic acid residues. Digestion of the total oligosaccharide mixture with the α-2,3-specific neuraminidase from Newcastle Disease Virus (29) resulted in the disappearance of oligosaccharide species H2 and H4 from the profile (Fig. 2C). The effect of Newcastle Disease Virus neuraminidase digestion on ISSA-HPLC-purified oligosaccharide H1 with specific oligosaccharide fractions and their native elution positions in all figures. A, 3H-oligosaccharide mixture released by alkaline degradation. B, products of C. perfringins neuraminidase digestion of oligosaccharide mixture. Similar digestion of the equimolar mixture of purified H2, H3, and H4 resulted in the conversion of all of these molecules to a species which co-eluted with H1 (not shown). C, products of α-2,3-specific Newcastle Disease Virus neuraminidase digestion of oligosaccharide mixture. D, products of digestion of ISSA-HPLC-purified oligosaccharide H4 by α-2,3-specific neuraminidase from Newcastle Disease Virus.

Analysis of Oligosaccharide H1—Based upon compositional data of the complete hinge region glycopeptide which indicated the presence of Gal and GalNAc in approximately equimolar ratios (Table II) and the finding that H1 was neutral by anion exchange HPLC, it was proposed that H1 was the NaB[3H]4-reduced derivative of Galβ1-3GalNAc, the predominant structure located in the hinge region of IgA (1). Digestion of ISSA-HPLC-purified oligosaccharide H1 with β-galactosidase (Jack bean meal) resulted in the formation of a product which co-migrated with authentic GalNAcol on both ISAA-HPLC and borate anion exchange HPLC (Fig. 3). This latter technique was employed as GalNAcol and GlcNAcol are not effectively resolved by ISAA-HPLC, whereas borate anion exchange HPLC readily separates these molecules (18). As noted in previous studies of Galβ1-3GalNAc (1, 30), digestion with this enzyme proceeds slowly and 4 days (with four additions of enzyme) were required to achieve the degree of completion observed.

The linkage position of Gal to GalNAcol in oligosaccharide...
The elution positions of the reduced structure of this disaccharide was Galβ1→3GalNAcol and the absence of a strong signal at m/e 174 and the presence of significant responses at m/e 88, 130, 187, 219, 276, 304, 378, 422, and 466 which were in ratios consistent with the fragmentation of a permethylated hexopyranosyl Dl-3 2-acetamido-2-deoxy-hexitol as described previously (26). The absence of a strong signal at m/e 174 and the presence of significant responses at m/e 304 and 378 ruled against the 14 linked compound.

The absence of a strong signal at m/e 174 and the presence of significant responses at m/e 304, 378 and 466 ruled against the presence of the 1–6 linked compound (26). Permethylated alditol acetates generated from oligosaccharide H1 by acetylation acid hydrolysis (23) and methanalysis (25) were also analyzed and resulted in the detection of terminal galactose and 3-monosubstituted N-acetylgalactosaminol, further confirming the structure assignment of this species as Galβ1→3GalNAcol.

**Analysis of Oligosaccharide H2**—The digestion by Newcastle Disease Virus neuraminidase of oligosaccharide H2 to a species which co-migrated with H1 (Fig. 2C) indicated that H2 was derived from H1 (Galβ1→3GalNAcol) by the addition of an α-2→3 linked neuraminic acid. Because the 3’ hydroxyl of the GalNAcol was already occupied by galactose in a glycosidic linkage, the neuraminic acid must have been linked to the 3’ hydroxyl of the galactose residue. Methylation analysis identified 3-monosubstituted galactose, 3-monosubstituted GalNAcol, and terminal N-acetylneuraminic acid. These results determined that the structure of oligosaccharide H2 was NeuAca2→3Galβ1→3GalNAcol.

**Analysis of Oligosaccharide H3**—Because oligosaccharide H3 was resistant to digestion with the α-2→3 specific neuraminidase from Newcastle Disease Virus (Fig. 1, C and D), it was proposed that H3 is derived from H1 (Galβ1→3GalNAcol) by the addition of 1 sialic acid residue linked in a manner other than 2,3. Methylation analysis identified terminal galactose, terminal N-acetylneuraminic acid, and 3,6-disubstituted N-acetylgalactosaminol. All of these results defined the structure of oligosaccharide H3 as Galβ1→3(NeuAca2→6)GalNAcol.

**Analysis of Oligosaccharide H4**—The digestion by Newcastle Disease Virus neuraminidase of oligosaccharide H4 to a species which co-migrated with H3 on ISSA-HPLC (Fig. 2D) indicated that H4 was derived from H3 (Galβ1→3(NeuAca2→6)GalNAcol) by the addition of a sialic acid residue linked α-2,3 to the terminal galactose of H3. Methylation analysis of oligosaccharide H4 identified terminal N-acetylneuraminic acid, 3-monosubstituted galactose, and 3,6-disubstituted N-acetylgalactosaminol. These results demonstrated that the structure of oligosaccharide H4 was NeuAca2→3Galβ1→3(NeuAca2→6)GalNAcol. It is not known why oligosaccharide H4 eluted later than a disialylated complex oligosaccharide on anion exchange HPLC (Fig. 1); however, the absence of more extensively substituted sugars in methylation analysis definitively ruled out the presence of additional sialic acid residues. Variability is often noted in the elution position of disialylated oligosaccharides on anion exchange HPLC (17). This may be related to differences in net carbohydrate composition (31) or subtle differences in the net charge of differently linked sialic acids at pH 4.0.

**Analysis of Fraction X**—Fraction X eluted in the position of a monosaccharide in ISSA-HPLC but did not co-migrate with any monosaccharide standard on borate anion exchange HPLC (Fig. 3). This species was not 3H-GalNAcol. Gas liquid chromatography-mass spectrometry of methylated and trimethylsilylated derivatives did not reveal the presence of any identifiable saccharide material. We therefore conclude that X is either a product of the alkaline-peeling reaction (32) or is a radioactive contaminant from the NaB[3H], reagent.

**Distribution of Oligosaccharides in the Hinge Region**—Defined glycopeptides encompassing either the entire hinge region or subfragments within it were subjected to alkaline degradation and the 3H-oligosaccharide products were analyzed by ISSA-HPLC. The relative compositions of oligosaccharide species H1, H2, H3, and H4 present on the different glycopeptides are displayed in Table III. Glycopeptides C6119–162, C6106–137, and complete IgD:WAH contained all of the hinge region glycosylation sites. Glycopeptide C6106–124 contained the glycosylated serine at C6109, and glycopeptide C6125–137 contained the glycosylated threonine residues at C6126, and 127 and C6131 and/or C6132 (14). A similar distribution of oligosaccharide species was present on all glycopeptides which indicated that all structures are likely to be found at each site and that there is little specificity of the hinge region.

The overall presence of sialylated species in ISAA-HPLC was consistent with the quantities of sialic acid found in carbohydrate analysis (Table I) and indicated that sialic acid was not lost nonspecifically during the purification of the hinge subfragments or during alkaline degradation. The presence of an O-glycosidically linked oligosaccharide at serine C6109, which had not been indicated by a conversion of 1 residue of serine to alanine after alkaline degradation (Table I), was confirmed by the detection of the full set of

**Table III**

**Distribution of oligosaccharides within hinge region**

| Glycopeptide | Oligosaccharide | H1 | H2 | H3 | H4 |
|--------------|-----------------|----|----|----|----|
| IgD:WAH      | 50              | 29 | 6  | 6  |
| C6106–137    | 39              | 32 | 16 | 13 |
| C6106–124    | 57              | 19 | 19 | 5  |
| C6125–137    | 60              | 23 | 12 | 5  |

**Fig. 3. Analysis of oligosaccharide H1.** An aliquot of purified oligosaccharide H1 was digested with β-galactosidase (jack bean meal). The products were analyzed by ISSA-HPLC on MicroPak AX-5 (A) and by borate anion exchange HPLC on DA-X8-11 (B) (18). Elution positions of the reduced forms of the indicated monosaccharides are displayed.
\(^3\)H-oligosaccharides liberated from glycopeptide C6106–125 (Table III). We presume that the unexpectedly elevated level of serine in the amino acid analysis following alkaline degradation was due to the presence of a serine-containing contaminant.

**Discussion**

A schematic representation of the constant region of the human 6 chain is depicted in Fig. 4. Emphasis has been placed on oligosaccharide structures and their localization. The predominant oligosaccharide species in the hinge region of IgD:WAH is the non-sialylated disaccharide Galβ1→3GalNAc. This structure comprises \(\approx 50\%\) of all oligosaccharides at each hinge region glycosylation site. The two monosialylated isomers NeuAcα2→3Galβ1→3GalNAc and Galβ1→3(NeuAcα2→6)GalNAc comprise \(\approx 40\%\), and the remaining \(\approx 10\%\) of the oligosaccharides have the structure NeuAcα2→3Galβ1→3(NeuAcα2→6)GalNAc. Although the sialylated O-linked oligosaccharides of the IgD hinge region have not previously been described on other human immunoglobulins, all of the oligosaccharide structures determined in this study have also been found on a variety of secreted and membrane associated glycoproteins. These include canine submaxillary mucin (33), bovine kininogen (34), and human erythrocyte sialoglycoprotein (35), among others (36).

In IgD:WAH, O-glycosidically linked oligosaccharides have been localized to serine C6109 and threonines C6126, 127, 128, and 132. It is uncertain whether both, or only one of the threonines at C6131 and C6132 are glycosylated (14). The amino acid sequence surrounding these oligosaccharides are (triplet rule) may serve to elicit the transfer of GalNAc to the serine and threonine residues of these sequences in human IgD as well as in other glycoproteins. The proposed rules are unlikely to be generally applicable for a number of reasons.

Based on the proposed distribution of the O-linked glycosylation sites in the hinge region of IgD:NIG-65, Takayasu et al. (38) have proposed two rules which designate particular amino acid sequences as generalized acceptor sequences for the O-linked glycosylation of glycoproteins. These rules propose that the specific amino acid sequences Ala-X-Ala-Ser or Ala-X-Ala-Thr-Thr (quintet rule) and Val-Pro-Thr (triplet rule) may serve to elicit the transfer of GalNAc to the serine and threonine residues of these sequences in human IgD as well as in other glycoproteins. The proposed rules are unlikely to be generally applicable for a number of reasons. IgD:WAH contains the same hinge region amino acid sequence as IgD:NIG-65 but is not glycosylated at all of the same hinge region positions (14). This indicates that the quintet and triplet sequences are not strict determinants of oligosaccharide localization. IgA1 contains an extensive degree of O-glycosylation in its hinge region; however, the amino acid sequences surrounding these oligosaccharides are predominantly a repeating pattern of Pro-Ser and none of the quintet or triplet sequences are present (1). There is also a considerable body of data available which indicates that in

![Fig. 4. Schematic representation of C6:WAH.](http://www.jbc.org/)

**Fig. 4.** Schematic representation of C6:WAH. Essential structural features of the heavy chain constant region of IgD:WAH are depicted with emphasis placed on oligosaccharide structure and localization (after Putnam et al. (45)). The GalNAc-rich segment of the hinge is shown in expanded view. Symbols (+) and (−) represent the highly charged segment of the hinge which is extremely sensitive to proteolytic degradation. Microheterogeneity of N- and O-glycosidically linked oligosaccharides is reflected by the presence or absence (±) of terminal sugar residues (see text). Asn 354, 445, and 496 refer to locations of the N-glycosidically linked oligosaccharides present on the δ chain of IgD:WAH which have been described previously (7). These residue numbers were calculated beginning at the NH₂ terminus of the δ chain. Their positions with respect to the first residue of the constant region is also indicated schematically below. TP refers to the tail piece of secreted form of IgD. Relative sizes of oligosaccharide units and protein domains are not drawn to scale.
a variety of glycoproteins which contain O-linked oligosaccharides, the glycosylated serine and threonine residues are not immediately surrounded by any specific set of amino acid residues (reviewed in Refs. 39–41). Aubert et al. (42) examined the amino acid sequences surrounding 9 different sites of O-glycosylation and did not find any characteristic amino acid sequence associated with the O-glycosylated residues. Aubert et al. (42), through the application of a computer program for the prediction of protein secondary structure based upon amino acid sequence data (43), also made the observation that each of the sites of O-glycosylation examined in their study was capable of participating in a β-turn structure. The authors concluded that the β-turn conformation served an important role in maintaining the accessibility of specific serine and threonine residues to the action of N-acetylgalactosaminyltransferase, the initial enzyme in the biosynthetic pathway of O-linked oligosaccharides (42). This is consistent with the fact that O-glycosylation is generally a late event in post-translational modification and occurs after proteins have attained a highly ordered structure (44). It is likely that accessibility of specific Ser and Thr residues to the N-acetylgalactosaminyltransferase as well as other higher order structural features will prove to be more critical determinants in the specific localization of O-linked oligosaccharides than will the presence of characteristic amino acid sequences as seen in N-glycans.

According to Putnam et al. (45) the hinge region of the δ chain differs from the hinges of γ and α in four notable characteristics: 1) its extreme length (~64 residues); 2) its division into a GalNAc-rich NH2-terminal half and a highly charged COOH-terminal half; 3) its composition and unusual half-cystine. The highly charged segment of the IgD hinge region serves to protect the high charge segment from proteolysis. According to Putnam et al. (45) postulates that each of the sites of O-glycosylation examined in their study was found on glycopeptides which encompassed either the glycosylated serine at C6109 or the glycosylated threonine residues between C6126 and C6192 (Table III). This heterogeneity of oligosaccharide structure can be expected to further accentuate the configurational disorder of the hinge region which has been predicted on the basis of amino acid sequence alone (14).

The absence of GalNAc alone or NeuAcα2→6GalNAc linked to serine or threonine provides suggestive evidence that the glycosylation pattern observed in IgD:WAH reflects biosynthetic variability in the activities of neuraminyl transferases upon an obligate Galβ1→3GalNAc-Ser/Thr substrate in these cells. It has been noted, however, that the 1,3 β-galactoside linkage in this structure is difficult to degrade under laboratory conditions (1, 30). This observation leaves open the possibility that more extensively sialylated oligosaccharides were initially synthesized and that the low level of disialylated oligosaccharides observed may be the result of glycosidases present in the serum of this myeloma patient. It will be necessary to perform biosynthetic studies with IgD plasmacytoma cells in vitro in order to ultimately resolve this issue.

The heterogeneity of oligosaccharide structure found in the hinge region of IgD:WAH as well as the ubiquitous presence of these structures on many glycoproteins suggest that the function of these glycans is not related to a particular mechanism involving the biological recognition of specific carbohydrate structures; however, this possibility has not been ruled out. The model of membrane IgD-mediated lymphocyte activation proposed by Putnam et al. (45) postulates that prior to antigen exposure, the GalNAc-rich portion of the hinge region serves to protect the high charge segment from proteolysis. Upon antigen binding, a conformational change would expose the high charge segment to enzymatic cleavage and ultimately result in blast transformation by one or more mechanisms (45). Testing of this hypothesis awaits determination of the structure of the oligosaccharides present on the membrane form of IgD and the performance of further immunological studies.

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