Possible Role for Peptide–Oligosaccharide Interactions in Differential Oligosaccharide Processing at Asparagine-107 of the Light Chain and Asparagine-297 of the Heavy Chain in a Monoclonal $IgG_1\kappa$†

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ABSTRACT: The carbohydrate attached at Asn-107 of the light chain of a human myeloma $IgG_1\kappa$ (Hom) was isolated and the structure determined by ^1H NMR. Two oligosaccharides were found corresponding to mono- and disialylated forms of the bisected biantennary class of glycopeptides. Both structures had Fucα1–6 linked to the GlcNAc residue attached to Asn and NeuNAc residues linked α2–6. Because of the unusual nature of these structures, the Asn-297 oligosaccharides of the same IgG were prepared from Fc fragments and heavy chains. Comparison of the structures of the latter glycopeptides with structures from the same site on a second human myeloma $IgG_1\kappa$ (Tem) showed them to be quite similar in that the majority of the structures were biantennary but not bisected. We suggest that the completely bisected nature of the light-chain oligosaccharides comes from a high level of activity of GlcNAc-T-III (the enzyme responsible for the attachment of the bisecting GlcNAc) in the cells producing the IgG. We suggest a mechanism for differential glycosylation between the Asn-107 and Asn-297 sites based on the stabilization of the Asn-297 oligosaccharide in a conformation with the torsional angle ω about the C5–C6 bond of the Manα1–6 linkage equal to −60°. It has previously been postulated that this conformation is not a substrate for GlcNAc-T-III [Brisson, J.-R., & Carver, J. P. (1983) *Can. J. Biochem. 61*, 1067–1078]. In support of this model are the X-ray crystallographic studies of the Fc fragment of human IgG from pooled serum [Deisenhofer, J. (1981) *Biochemistry 20*, 2361–2370]. In these crystals the conformation for the oligosaccharide at the Asn-297 site was found to be in the ω = −60° form.

In an earlier paper (Savvidou et al., 1981), a human IgG_1k monoclonal protein (Hom) was described in which some of the L chains were glycosylated at Asn-107 within the J region. The absence of carbohydrate on a proportion of the chains was not due to a lack of the appropriate acceptor sequence but may have reflected a competition between protein folding and the transfer of core sugars from dolichol pyrophosphate oligosaccharide. In this paper, we describe the determination of the structure of the carbohydrate of the L chain and a comparison with the structure of the carbohydrate present on the $C_{\gamma 2}$ domain from the same protein. The latter structures were found to be similar to those determined for human myeloma IgG(Tem) (Grey et al., 1982), whereas the carbohydrate from the L chain of IgG(Hom) was found to have a relatively rare structure belonging to the sialylated bisected biantennary class.

Materials and Methods

Proteins, Polypeptides, and Fragments. IgG(Hom), its H and L chains, and Fab and Fc fragments were prepared as described previously (Savvidou et al., 1981). However, the H chains were separated from L chains on a Bio-Gel P-60 column (5 × 95 cm) equilibrated in 1 M acetic acid/25 mM NaCl. This column was used instead of Sephadex in order to avoid possible contamination with phenol/sulfuric-positive material from the column.

Preparation of Glycopeptides. Essentially similar methods were used to prepare glycopeptides from the L chains, H chains, and Fc fragments of IgG(Hom). L chain at approximately 10 mg/mL in 0.1 M Tris-HCl buffer, pH 8.0/2 mM $CaCl_2$ was digested with 1% (w/w) Pronase (Calbiochem) at 37 °C. Toluene was used as a bacteriocide. Pronase was added twice at 24-h intervals, and the pH was readjusted to 8.0. After a total digestion time of 48 h, the digestion mixture was concentrated by rotary evaporation at 40 °C to approximately half its original volume and applied on a Bio-Gel column. Under these conditions, there is no cleavage of NeuNAc linkages.

For the L chain glycopeptide preparation, the concentrated digest was loaded onto a 2.5 × 63 cm column of Bio-Gel P-4 (Bio-Rad Biochemicals) in distilled water containing 0.02% NaN_3. The digestion products were eluted at a rate of 10–15 mL/h, and the eluent was monitored by absorption at 280 and/or 230 nm, as well as colorimetrically for neutral sugar by the phenol/sulfuric acid method of Dubois (1956). The carbohydrate-containing pool was concentrated and lyophilized. The L-chain glycopeptides were further fractionated by chromatography on a column of Bio-Gel P-6 in distilled water/0.02% NaN_3 with an elution rate of 2–3 mL/h. Small-volume fractions (~1 mL) were collected, and the effluent was monitored as described above. Two major peaks containing carbohydrate, designated L-I and L-II (Figure 1A), were pooled, lyophilized, and prepared for NMR.

The H-chain sample (800 mg) was digested with Pronase 3 times, enzyme being added at 24-h intervals. The digest was lyophilized and applied to a column of Bio-Gel P-6 desalting gel (2.5 × 95 cm). Two phenol/sulfuric-positive peaks were obtained, pooled, and designated H-I and H-II (Figure 1B). The two pools were further purified by removing most of the contaminating peptides by passage through a Dowex 50W-X8 column equilibrated in 10 mM sodium acetate, pH 3.2. The phenol/sulfuric-positive pools from the Dowex profile were then desalted on a column of Bio-Gel P-2. Further fractionation of H-II was carried out on a Con A-Sepharose column as described in Grey et al. (1982). The material coming through the column was divided into two pools H-IIA and H-IIB corresponding to the leading and trailing portions, respectively.

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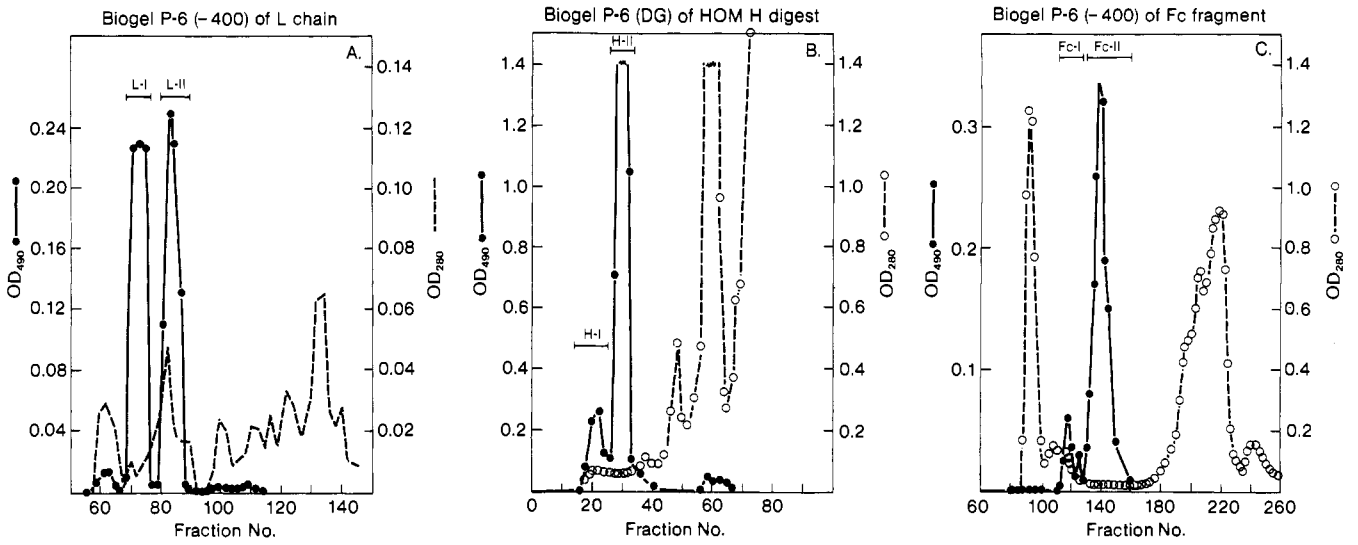


FIGURE 1: Bio-Gel P-6 profiles of Pronase digests: the solid lines correspond to OD_{490} from the phenol/sulfuric assay for neutral sugar, and the dotted line corresponds to OD_{280}, indicating the presence of peptides. (A) L chains, 1.35 mL/fraction, total column volume 320 mL; (B) H chains, 6.7 mL/fraction, total column volume 460 mL; (C) Fc fragments, 1.54 mL/fraction, total column volume 460 mL.

Hom Fc fragment (221 mg) at a concentration of 8.5 mg/mL was Pronase-digested as previously described and then applied on a Bio-Gel P-6 (−400 mesh) column (2.5 cm × 94 cm) and eluted in distilled H_2O containing 0.02% NaN_3. Two sugar-containing peaks were obtained (Figure 1C) and designated Fc-I and Fc-II. Both samples were lyophilized from D_2O and characterized by nuclear magnetic resonance. Peptide fragments were removed from Fc-I on a Dowex 50W-X8 column equilibrated in 1 mM sodium acetate, pH 4.0.

Nuclear Magnetic Resonance Spectroscopy. Samples were desalted if necessary on Bio-Gel P-2 or P-6 columns, lyophilized, and exchanged twice in 99.8% D_2O (Bio-Rad or Merck Sharp & Dohme Canada Ltd.). When preliminary NMR spectra showed evidence of paramagnetic metal ion contamination, samples were run through a Chelex 100 column (100–200 mesh, Bio-Rad). Spectra were obtained at both 24 and 70 °C on a Nicolet 360-MHz high-resolution spectrometer operated with quadrature detection in the Fourier-transform mode and located at the Toronto Biomedical NMR Centre. Adequate signal to noise was obtained with 32 scans of 16K data points at a sweep width of ±1500 Hz with the transmitter centered on the HDO resonance. The resulting digital resolution was 0.375 Hz per data point in the transformed spectrum. A pulse width of 8 μs (90° pulse) was used together with a delay of 5 s between scans; the latter was required in order to suppress the residual HDO resonance sufficiently to observe the anomeric proton resonances. Chemical shifts were calculated relative to an internal acetone reference and expressed relative to external 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) by using a value of 2.225 ppm for the chemical shift of acetone at both 24 and 70 °C.

Amino Acid Analyses of L-Chain Glycopeptides. Small samples of L-I and L-II were lyophilized and then hydrolyzed for 24 h at 110 °C in 6 N constant-boiling HCl in an evacuated sealed tube. HCl was removed in a desiccator over KOH. Sodium citrate buffer, pH 2.2, was added to the samples, which were then chromatographed on a Minnie amino acid analyzer column as described by Moore & Stein (1963).

Results

Light-Chain Glycopeptides. The NMR spectra obtained for L-I and L-II in the region of anomeric hydrogen resonances are shown in Figure 2. The chemical shift data on which the

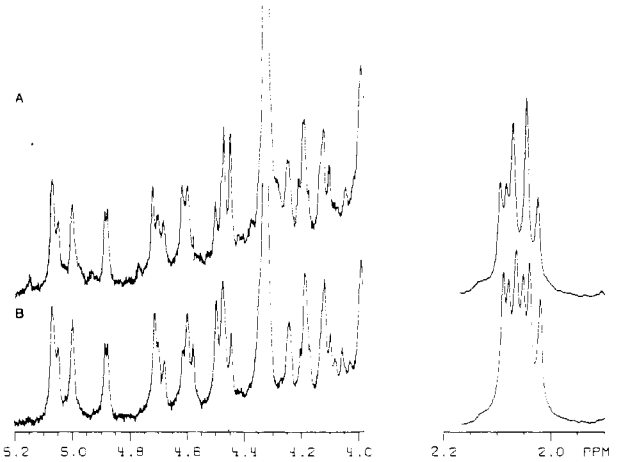


FIGURE 2: Regions of C1-H and *N*-acetyl resonances in the 360-MHz NMR spectra of (A) L-I and (B) L-II.

structural identification is based are shown in Table I. The chemical shifts of the resonances from the C1-H and C2-H of the mannosyl residues immediately establish that both these glycopeptides belong to the bisected biantennary complex class (Carver & Grey, 1981). The 3.5-Hz doublet at ~4.88 ppm (C1-H) and the 6.5-Hz doublet at ~1.2 ppm (C6 methyl) both indicate the presence of a Fucα1–6 residue. The chemical shift of the C1-H of the core GlcNAc, at 23 °C, is 4.678 ppm; this value is characteristic of a structure in which a fucose residue is attached to the 6 position of Asn–GlcNAc (Carver & Grey, 1981). Chemical shift data for bisected biantennary oligosaccharides (Strecker et al., 1977) and bisected triantennary glycopeptides (Dorland et al., 1979) have been published previously; however, in all these cases the mannosyl core structure was only substituted by GlcNAc, and no Gal or NeuNAc residues were present. The multiplets at ~4.6 ppm (C1-H of GlcNAcβ1–2) and ~4.5 ppm (C1-H of Galβ1–4) confirm that for the L-chain glycopeptides the mannose core is substituted with lactosamine units. Furthermore, multiplet resonances occur at ~2.69 and ~1.68 ppm (70 °C) (these are partially obscured by the β-CH_2 resonances of Asn and Arg, respectively), which can be assigned to the H3(e) and H3(a) of NeuNAcα2–6 residues linked to the Galβ1–4 units. Thus both glycopeptides are α2–6 sialylated. From a consideration of the resonances in three different chemical shift

Table I: Chemical Shifts for Mannose C1-H and C2-H of L-I, L-II, H-IIA, and H-IIB

residue	hydrogens	chemical shift (ppm)[a] L-I	L-II	H-IIA	H-IIB[b]
Manα1–3	C1-H/C2-H	5.070/4.261	5.074/4.263	5.055/4.254	5.057/4.253
		5.069/4.243	5.071/4.243	5.056/4.236	5.057/4.240
Manα1–6[c]		5.015/~4.15	5.027/~4.14	5.016/~4.14	5.013/~4.14
		5.000/~4.12	4.999/~4.12	4.992/~4.12	4.992/~4.12
Manβ1–4		4.688/4.186	4.692/4.189	4.688/4.177	4.685/4.173
		4.715/4.187	4.711/4.188	4.702/4.176	4.705/4.178
Fucα1–6	C1-H/C6 Me	4.88/1.201	4.884/1.200	4.880/1.199	4.881/1.200
		4.881/1.212	4.882/1.210	4.878/1.209	4.881/1.210
Asn-GlcNAc	C1-H/NAc[d]	5.056/2.011	5.062/2.012	5.070/2.010	5.070/2.009
		5.058/2.019	5.066/2.019	5.064/2.019	5.088/2.019
core GlcNAc		4.677/2.100	4.679/2.095	4.673/2.093	4.674/2.095
		4.689/2.090	4.691/2.088	4.685/2.087	4.687/2.088
GlcNAcβ1–2 (3-arm)		4.619/2.080	4.617/2.078	4.579/2.062	4.556/2.065
		4.603/2.078	4.606/2.078	4.582/2.062	4.555/2.068
GlcNAcβ1–2 (6-arm)		4.597/2.066	4.588/2.044	4.579/2.040	4.579/2.039
		4.603/2.066	4.587/2.050	4.582/2.048	4.584/2.038
bis(GlcNAcβ1–4)		4.464/2.058	4.467/2.057	4.466/2.056	4.465/2.055
		4.487/2.066	4.484/2.063	4.480/2.062	4.483/2.049
Galβ1–4 (3-arm)	C1-H	4.442	4.442	4.474	
		4.455	4.459	4.480	
Galβ1–4 (6-arm)	C1-H	4.442	4.478	4.474	4.479
		4.455	4.484	4.480	4.483
NeuNAcα2–6	NAc	2.030	2.031		
		2.040	2.039		

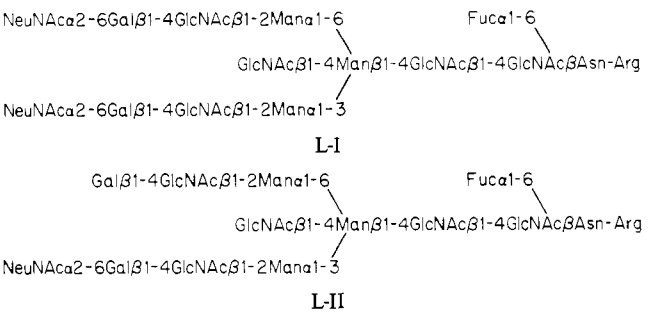
[a] The chemical shifts are quoted for 23 (upper line) and 70 °C (lower line) for each residue. [b] The assignments of the NAc resonances differ from those given in Grey et al. (1982) for the same compound isolated from IgG(Tem). These reassignments are necessary to reconcile the two sets of data. [c] The position of the C2-H resonance is obscured by the Fuc C5-H and other resonances. [d] The assignments of the NAc resonances have been made on the basis of chemical shift correlations since no direct method is available at present. This approach assumes minimum perturbation of chemical shifts in a family of closely related compounds and may be open to error if dramatic conformational alterations occur.

regions (the region of anomeric resonances, the region of *N*-acetyl resonances, and the intensities at ~2.67 and ~1.72 ppm), it can be concluded that L-I is fully sialylated and L-II has only one sialylated lactosamine unit, that attached to the Manα1–3 residue. These points are discussed in more detail below.

In the region of anomeric resonances of L-II at 70 °C (Figure 2), two chemically shifted doublets for the C1-H of each Galβ1–4 (4.456 and 4.484 ppm) and GlcNAcβ1–2 (4.606 and 4.587 ppm) residue are observed. However, in the spectrum of L-I, the GlcNAcβ1–2 anomeric hydrogen resonances are superimposed at 4.603 ppm and those of the Galβ1–4 at 4.455 ppm (the doublet at 4.487 ppm arises from the bisecting GlcNAc C1-H), indicating that the two lactosamine units in L-I have become equivalent. That this equivalence arises from both being NeuNAcα2–6 substituted becomes evident when the intensities of the NeuNAc H3(a), H3(e), and *N*-acetyl resonances are compared in the spectra of L-I and L-II.

For L-I at 70 °C, there are five resolved peaks in the *N*-acetyl region, with two (2.040 and 2.066 ppm) double the intensity of the others. However, for L-II there are six approximately equal intensity peaks with a new *N*-acetyl resonance appearing at 2.050 ppm and a reduction in intensity of the 2.040 and 2.066 ppm peaks to half the intensities found in the spectrum of L-I. Similarly, the intensities of the H3(a) and H3(e) resonances of NeuNAc (~2.697 and ~1.68 ppm) are reduced in the spectrum of L-II compared to that of L-I. The conclusion that the Manα1–6 arm lacks a terminal NeuNAcα2–6 residue in L-II comes from nuclear Overhauser effect (NOE) experiments and a comparison of the chemical shifts for the C1-H resonances of Manα1–6 and Manα1–3 between L-I and L-II. When an NOE experiment was performed on L-II in which the C1-H resonance of the Manα1–3 was irradiated (5.074 ppm), an NOE was observed on the C1-H resonances of the β1–2-linked GlcNAc residue at 4.619 ppm. Similarly, an experiment in which the Manα1–6 C1-H

Chart I



resonance at 5.027 ppm was irradiated yielded an NOE at 4.588 ppm. Thus, the resonance at 4.619 ppm can be assigned to the GlcNAc linked to the Manα1–3 residue and that at 4.588 ppm to the GlcNAc linked to the Manα1–6 (Brisson & Carver, 1983d). Since NeuNAcα2–6 substitution on the terminal Gal is known to cause a downfield shift of 0.024 ppm on the GlcNAcβ1–2 C1-H resonance in unbisected biantennary glycopeptides (Grey et al., 1982) and the anomeric resonance of the 3-arm GlcNAc is shifted 0.031 ppm downfield relative to that of the 6-arm in L-II, it is reasonable to place the terminal NeuNAc residue on the 3-arm. This assignment is supported by the mannosyl anomeric chemical shifts. The chemical shifts of C1-H of Manα1–3 are essentially identical in both compounds (5.070 and 5.074 ppm, at 20 °C, for L-I and L-II, respectively) whereas those for the corresponding Manα1–6 residues are 5.015 and 5.027 ppm, respectively.

Resonances found at ~2.7 ppm in the spectrum obtained at 70 °C were assigned to the β-CH_2 of the Asn, those at ~1.65, ~1.87, and ~3.25 ppm to the β-, γ-, and δ-CH_2 groups, respectively, of Arg, and the minor components at ~0.9 ppm to the CH_3 groups of Ile. Amino acid analysis confirmed the presence of an Arg residue in a ratio of 1:1 with Asn; small amounts of Ile and Glu were also detected. These results are consistent with the sequence at the glycosylation site being Glu-Ile-Asn-Arg-Thr (Savvidou et al., 1981).

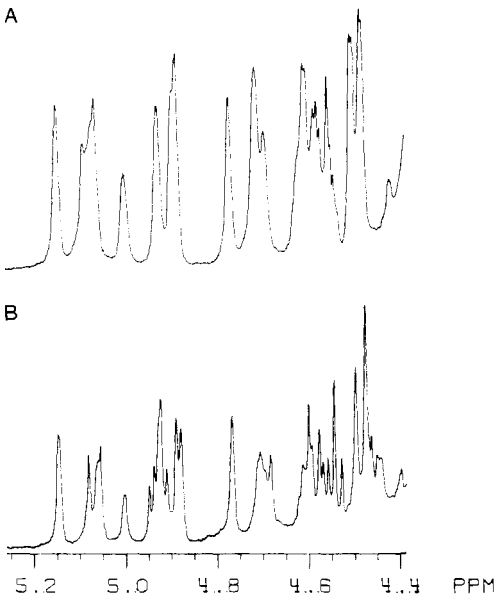


FIGURE 3: Regions of the 360-MHz NMR spectra containing the C1-H resonances for the pools of neutral glycopeptides from Pronase digests of (A) H chains of IgG(Hom) and (B) Fc of IgG(Hom).

Thus the two glycopeptides were assigned the structures shown in Chart I. The presence of a bisecting GlcNAc moiety is not a common phenomenon with IgG glycopeptides but has been previously described (Baenziger & Kornfeld, 1974). In a recent study of the glycopeptide structures attached to the H chain of human myeloma IgG(Tem), Grey et al. (1982) found unbisected biantennary complex structures with only a small amount (5%) of bisected biantennary complex present. Furthermore, no fully sialylated species were found. Thus, the structures reported above for the L chain of IgG(Hom) are unusual in two respects: (i) the presence of only bisected species and (ii) the high degree of sialylation. These differences could arise from elevated levels of GlcNAc transferase III (Narasimhan, 1982) and α(2–6)sialyl transferase in the cells producing the IgG. If so, the glycopeptides attached at Asn-297 of the heavy chain might also be expected to be a bisected biantennary complex with a high degree of sialylation. Alternatively, the L-chain structures might arise from a specific interaction between the polypeptide conformation and the transferases/glycosidases of the biosynthetic pathway. In this case, the structures at Asn-297 might be expected to resemble those of the heavy chain of IgG(Tem). To resolve these possibilities, the glycopeptides from the isolated heavy chain of IgG(Hom) were prepared and their structures determined by NMR.

Heavy-Chain Glycopeptides. The digest of the heavy chains resulted in two phenol/sulfuric-positive peaks on Bio-Gel P-6DG designated H-I and H-II (Figure 1B) in a ratio of 1:7. Examination of the NMR spectra of the two fractions showed that they corresponded to sialylated (H-I) and neutral biantennary glycopeptides (H-II). Anomeric resonances characteristic of bisected structures were present only in the spectrum of the neutral fraction (Figure 3A). The ratio of bisected to unbisected structures was estimated from the intensity of the resonance at ~5.01 ppm (Manα1–6 in bisected structures) relative to that at ~5.15 ppm (Manα1–3 in unbisected structures) and was found to be 1:2. Thus a greater proportion of the neutral structures are bisected than was found for IgG(Tem), where the corresponding ratio was 1:13. When the overall fraction of bisected structures is calculated by taking into account the unbisected sialylated species, the percent bisected structures at the Asn-297 site is found to be 5% for IgG(Tem) (Grey et al., 1982) and 27% for IgG(Hom).

Preliminary experiments cited below point to the fact that the H chain is normally glycosylated; that is, it possesses one oligosaccharide moiety per chain. First, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) gels showed only one band for the H chain. Judging from the results of SDS–PAGE on Fab in which two bands were seen [see Savvidou et al. (1981)], it seems reasonable to assume that if any major differences in H chain existed due to glycosylation, it would be obvious on the gels. Second, neutral sugar determination of H-chain samples by the phenol/sulfuric method (Dubois et al., 1956) gave an average of ~2.1% carbohydrate, which corresponds to one oligosaccharide moiety per H chain. The amount of glycopeptide obtained from a Pronase digest of H chain also suggests a 1:1 ratio. However, to confirm that the increased heterogeneity in heavy-chain structures for IgG(Hom) arises at a single glycosylation site, the glycopeptides from the Fc fragment were prepared. Two phenol/sulfuric-positive pools were obtained in the same way as for the H chains and their NMR spectra compared to those for the corresponding pools for the heavy-chain digest. Identical mixtures of structures were present as is illustrated by the comparison of the spectra for the anomeric regions of the two neutral pools shown in Figure 3. The fractions from the neutral pool of heavy-chain glycopeptides that came through Con A–Sepharose (H-IIA and H-IIB) were identified by ^{1}H NMR (Table I) to be the bisected structures GG(Gn) and GGn(Gn), respectively [in the nomenclature of Grey et al. (1982)].

Discussion

On those Hom L chains that were glycosylated (67%), the oligosaccharides were found to be entirely bisected biantennary complex structures, whereas the major H-chain oligosaccharides were normal biantennary complex structures showing a degree of heterogeneity with respect to terminal sugar attachment similar to that observed for another monoclonal IgG_1 molecule, IgG(Tem) (Grey et al., 1982). Earlier results from several studies of Hom IgG_1 chains and fragments suggested a monoclonal cell population was responsible for the production of the immunoglobulin (Savvidou et al., 1981). A kinetic mechanism involving competition between folding of the nascent polypeptide and attachment of core sugars was suggested to explain why one-third of the L-chain molecules was not glycosylated. Glycosylation of the L chains occurs at the initial stage of peptide synthesis (Bergman & Kuehl, 1978). Thus, if the precursor high-mannose intermediate is attached to the nascent L chain, then it may be processed in the later stages to yield a completed N-linked complex oligosaccharide; if no high-mannose oligosaccharide is attached at this stage, then no glycosylation will occur.

However, the relative rates of sugar attachment, peptide folding, and release from the polyribosome can only account for the presence or absence of an oligosaccharide at a glycosylation site. The differences between the oligosaccharide structures found at Asn-107 of the L chain and Asn-297 of the H chain must therefore arise through some different mechanism. Evidence has accumulated from several studies (Schachter & Roseman, 1980) that the enzymes responsible for the later stages of processing are located in the Golgi apparatus. At this point in the pathway, it would appear that the H and L chains are already covalently associated (Bergman & Kuehl, 1979). Thus, the oligosaccharides at the glycosylation sites of both the L and H chains should encounter the same array of processing transferases and/or glycosidases. The greater percentage of bisected structures at the H-chain site

in IgG(Hom) suggests that the clone of cells responsible for its synthesis may have a higher activity of GlcNAc-T-III than that responsible for the synthesis of IgG(Tem); however, the differences in the proportion of bisected structures occurring at the L-chain and H-chain glycosylation sites require further explanation.

The recent studies of Narasimhan (1982) have shown that GlcNAc-T-III (the enzyme that adds the bisecting GlcNAc) can act on several intermediate structures in the biosynthetic pathway. For structures destined to become biantennary complex, GlcNAc-T-III can act at any point after the combined action of GlcNAc-T-I and mannosidase II but must act before galactosylation of the 3-arm has occurred. This result presents a problem for any model that attempts to account for the difference in H- and L-chain structures on the basis of simple accessibility arguments. The majority of H-chain oligosaccharides are not bisected, yet they are extensively galactosylated. Thus the presence of unbisected structures at Asn-297 of the H chain cannot be attributed to the oligosaccharide being "buried" at this point in the pathway since it clearly is "accessible" to the Gal-T's that act further along. Furthermore, the fact that the L-chain oligosaccharides are fully bisected establishes that GlcNAc-T-III is sufficiently active to compete with galactosylation at the L-chain site. Why then are the H-chain structures not also all bisected? One possibility is that the three-dimensional surfaces formed by the oligosaccharide and the polypeptide chain at the two sites are sufficiently different that at the H-chain site that part of the oligosaccharide recognized by GlcNAc-T-III is relatively inacessible whereas that recognized by the Gal-T's remains exposed. At the L-chain site, on the other hand, both portions of the oligosaccharide must be exposed since fully bisected, fully galactosylated structures are found exclusively.

On the basis of recent substrate specificity studies (Narasimhan, 1982; Gleeson & Schacter, 1983) and a knowledge of the three-dimensional structure of the substrates (Brisson & Carver, 1983a,b), we have suggested (Brisson & Carver, 1983c) that part of the GlcNAc-T-III substrate interaction involves binding to the GlcNAcβ1–2Manα1–3Manβ1–4 segment common to all its substrates. Furthermore, because bisected biantennary complex structures were found with the Manα1–6 arm exclusively in the orientation corresponding to $\omega = 180°$, whereas the corresponding unbisected structures were in equilibrium between two forms with $\omega = -60$ and 180°, we suggested that only the three-dimensional structure with $\omega = 180°$ was a substrate for GlcNAc-T-III (Brisson & Carver, 1983c).

The three-dimensional structure of the Fc fragment from a human immunoglobulin, determined by X-ray diffraction studies (Deisenhofer, 1981), provides an opportunity to examine these suggestions more closely since the electron density for the oligosaccharide at Asn-297 is clearly resolved. The biantennary complex oligosaccharide is stabilized in the form with $\omega = -60°$ through interactions between the protein and the terminal Gal residue of the Manα1–6 arm. From the argument given above, this form of the oligosaccharide would not be expected to be a substrate for the GlcNAc-T-III, *regardless of accessibility*. It seems likely, therefore, that the minor fraction of biantennary complex structures that are bisected derives from oligosaccharides that had a bisecting GlcNAc added at an earlier point in the pathway [e.g., at the GnGn stage, in the nomenclature of Schachter et al. (1983)], before the oligosaccharide interaction with the protein became possible.

Thus, IgG(Hom) provides an excellent example of one mechanism whereby interaction between the peptide moiety of a glycoprotein could influence the class of oligosaccharide structures that will be found at a particular glycosylation site. This mechanism demonstrates a more subtle degree of control of specificity than a simple model of limited accessibility (Hsieh et al., 1983; Trimble et al., 1983) in that the latter terminates processing at some point in the pathway, whereas in IgG(Hom) the oligosaccharide/protein interaction at Asn-297 stabilizes a three-dimensional structure that is not a substrate for GlcNAc-T-III, thus drastically reducing the occurrence of bisected structures compared to the L-chain site while permitting all subsequent processing steps to proceed.

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Registry No. L-I, 90623-24-8; L-II, 90605-41-7; Asn, 70-47-3.

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