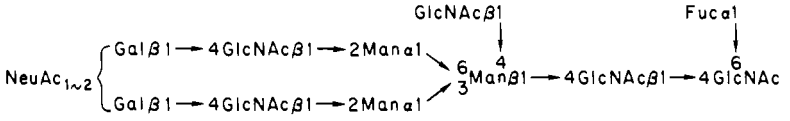
Stanley, P. (1980) in *The Biochemistry of Glycoproteins and Proteoglycans* (Lennarz, W. J., Ed.) pp 161–189, Plenum Press, New York.
Tabas, I., & Kornfeld, S. (1978) *J. Biol. Chem. 253*, 7779–7786.
Tai, T., Yamashita, K., Ito, S., & Kobata, A. (1977) *J. Biol. Chem. 252*, 6687–6694.
Tulsiani, D. R. P., & Touster, O. (1983) *J. Biol. Chem. 258*, 7578–7585.
Tulsiani, D. R. P., Harris, T. M., & Touster, O. (1982) *J. Biol. Chem. 257*, 7936–7939.
van Halbeek, H., van Beek, W. P., Blanken-Aarsen, G. J., & Vliengenthart, J. F. G. (1983) in *Proceedings of the International Symposium on Glycoconjugates, 7th* (Chester, M. A., Heinegard, D., Lundblad, A., & Svensson, S., Eds.) pp 864–865, Rahms i Lund, Sweden.
Varki, A., & Kornfeld, S. (1980) *J. Biol. Chem. 255*, 10847–10858.
Varki, A., & Kornfeld, S. (1983) *J. Biol. Chem. 258*, 2808–2818.
Vella, G., Paulsen, H., & Schachter, H. (1984) *Can. J. Biochem. Cell Biol. 62*, 409–417.
Vischer, P., & Hughes, R. C. (1981) *Eur. J. Biochem. 117*, 275–284.
Vliengenthart, J. F. G., van Halbeek, H., & Dorland, L. (1981) *Pure Appl. Chem. 53*, 45–77.
Vliegenthart, J. F. G., Dorland, L., & van Halbeek, H. (1983) *Adv. Carbohydr. Chem. Biochem. 41*, 209–374.

Structures of the Carbohydrate Moieties of Two Monoclonal Human λ-Type Immunoglobulin Light Chains†

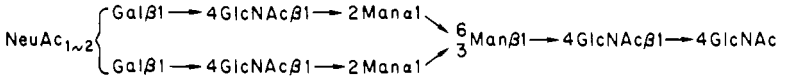
Takashi Ohkura, Takashi Isobe,‡ Katsuko Yamashita, and Akira Kobata*

Department of Biochemistry, Kobe University School of Medicine, Chuo-ku, Kobe, Japan
Received July 27, 1984

ABSTRACT: Human Bence Jones proteins of λ type, Wh and Nei, both of which belong to subgroup II, contain an asparagine-linked sugar chain. Their carbohydrate moieties were liberated as oligosaccharides by hydrazinolysis and labeled by reduction with NaB^3H_4 after N-acetylation. Structural studies of each oligosaccharide by sequential exoglycosidase digestion in combination with methylation analysis revealed that Wh λ contains the mono- and disialylated oligosaccharides



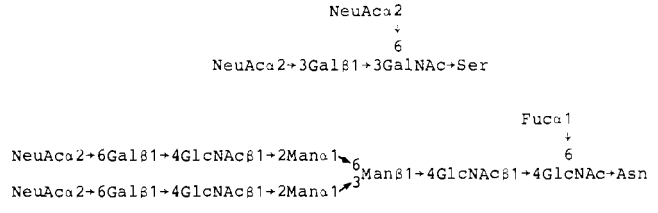
while Nei λ contains the two acidic oligosaccharides



These oligosaccharides are different from the oligosaccharides found in another λ-type Bence Jones protein, Sm λ, by Chandrasekaran et al. [Chandrasekaran, E. V., Mendicino, A., Garver, F. A., & Mendicino, J. (1981) *J. Biol. Chem. 256*, 1549–1555] and Garver et al. [Garver, F. A., Chang, L. S., Kiefer, C. R., Mendicino, J., Chandrasekaran, E. V., Isobe, T., & Osserman, E. F. (1981) *Eur. J. Biochem. 115*, 643–652].

Each of the heavy chains of human immunoglobulin G (IgG) contains an asparagine-linked sugar chain in its Fc portion (Clamp et al., 1964). Although the light chains usually lack carbohydrate, some light chains of human myeloma proteins were reported to contain sugar chains (Abel et al., 1968; Spiegelberg et al., 1970). The structure of the asparagine-linked sugar chain in Sm λ, one such carbohydrate-containing Bence Jones protein, was elucidated as shown in Chart I by Chandrasekaran et al. (1981) and Garver et al. (1981). By comparing the fractionation patterns of oligosaccharides released from 10 different IgG myeloma proteins by Bio-Gel P-4 column chromatography, we found that a variety of oligosaccharide patterns was obtained from these glycoproteins (Mizuochi et al., 1982). The carbohydrate-containing Bence Jones (BJ) proteins were reported to have different sugar contents (Abel et al., 1968; Spiegelberg et al., 1970). In view of recent reports that indicate a functional role

Chart I: Proposed Structures of Sugar Chains Found in BJ Protein, Sm λ, by Chandrasekaran et al. (1981) and Garver et al. (1981)



† This work has been supported in part by Grant-in-Aids for Scientific Research, the Ministry of Education, Science and Culture of Japan, and by a research grant from Yamada Science Foundation.
* Address correspondence to this author at the Department of Biochemistry, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108, Japan.
‡ Present address: Department of Medicine, Third Division, Kobe University School of Medicine, Chuo-ku, Kobe 650, Japan.

0006-2960/85/0424-0503$01.50/0

for the sugar chains in IgG (Ciccimarra et al., 1976; Huber et al., 1976; Nose & Wigzell, 1983), information concerning the structure of the sugar moieties of a range of carbohydrate-containing BJ proteins is important, although the incidence of sugar-containing BJ proteins is low.

In this paper, we report the structures of the sugar chains of two BJ proteins, Wh λ and Nei λ. The glycoproteins have a sugar chain in their variable regions at a different location from that in Sm λ, although the amino acid sequences of Wh λ, Nei λ, and Sm λ were classified into subgroup II (Garver & Hilschmann, 1972; Kiefer et al., 1980; Garver et al., 1981).

Experimental Procedures

Carbohydrate-Containing BJ Proteins. Three carbohydrate-containing BJ proteins, Sm λ, Wh λ, and Nei λ, were isolated from the urine of patients with a plasma cell dyscrasia (monoclonal gammapathy) as described by Isobe & Osserman (1974) and Garver & Hilschmann (1972).

Oligosaccharides. NeuAcα2→6Galβ1→4GlcNAcβ1→2Manα1→6(NeuAcα2→6Galβ1→4GlcNAcβ1→2Manα1→3)Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$[1] (NeuAc$_2$·Gal$_2$·GlcNAc$_2$·Man$_3$·GlcNAc·GlcNAc$_{OT}$) and NeuAcα2→6Galβ1→4GlcNAcβ1→2Manα1→6(Galβ1→4GlcNAcβ1→2Manα1→3)Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$ (NeuAc·Gal$_2$·GlcNAc$_2$·Man$_3$·GlcNAc·GlcNAc$_{OT}$) were obtained by hydrazinolysis of human transferrin (Spik et al., 1975). Manα1→6 (Manα1→3)Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$ (Man$_3$·GlcNAc·GlcNAc$_{OT}$) and Manβ1→4GlcNAcβ1→4GlcNAc$_{OT}$ (Man·GlcNAc·GlcNAc$_{OT}$) were prepared from NeuAc$_2$·Gal$_2$·GlcNAc$_2$·Man$_3$·GlcNAc·GlcNAc$_{OT}$ by sequential digestion with sialidase, diplococcal β-galactosidase, diplococcal β-*N*-acetylhexosaminidase, and jack bean α-mannosidase. GlcNAcβ1→2Manα1→6(GlcNAcβ1→4)(Manα1→3)Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc$_{OT}$ (GlcNAc·Man$_2$·GlcNAc·Man·GlcNAc·Fuc·GlcNAc$_{OT}$), Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc$_{OT}$ (Man$_3$·GlcNAc·Fuc·GlcNAc$_{OT}$), and Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc$_{OT}$ (Man·GlcNAc·Fuc·GlcNAc$_{OT}$) were prepared from α-fetoprotein purified from a human yolk sac tumor as previously described (Yamashita et al., 1983b).

Chemicals and Enzymes. NaB^3H_4 (348 mCi/mmol) was purchased from New England Nuclear, Boston, MA, and NaB^2H_4 (98%) from Merck Co., Darmstadt, G.F.R. *Arthrobacter ureafaciens* sialidase was purchased from Nakarai Chemicals, Ltd., Kyoto. β-Galactosidase and β-*N*-acetylhexodaminidase were purified from the culture fluid of *Diplococcus pneumoniae* (Glasgow et al., 1977). Another β-*N*-acetylhexosaminidase and α-mannosidase were purified from jack bean meal (Li & Li, 1972). Snail β-mannosidase (Sugahara et al., 1972) and *Charonia lampas* α-L-fucosidase (Nishigaki et al., 1974) were kindly supplied by Seikagaku Kogyo Co., Tokyo.

Isolation of Carbohydrate Moieties of BJ Proteins. Each of the BJ proteins (10 mg) was heated in 0.5 mL of anhydrous hydrazine in a sealed tube at 100 °C for 8 h, and the released oligosaccharides were completely N-acetylated with acetic anhydride as reported previously (Takasaki et al., 1982). One-tenth of each oligosaccharide fraction was reduced with 240 nmol (83 μCi) of NaB^3H_4 in 200 μL of 0.08 N NaOH at 30 °C for 4 h. The remainder was reduced with 2 mg of NaB^2H_4 under the same conditions. The reduction products were purified as described in a previous paper (Endo et al., 1979). The yields of radioactive oligosaccharides from Wh λ and Nei λ were 6.99×10^5 and 7.66×10^5 cpm, respectively.

[1] Subscript OT is used in this paper to indicate NaB^3H_4-reduced sugars. All sugars mentioned in this paper were of D configuration, except for fucose which had an L configuration.

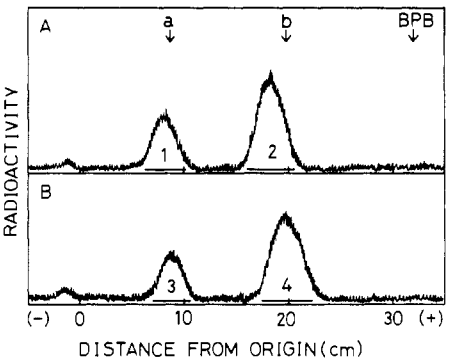


FIGURE 1: Paper electrophoresis of oligosaccharides liberated from Wh λ (A) and Nei λ (B) by hyrazinolysis. After reduction with NaB^3H_4, the oligosaccharide mixtures were subjected to paper electrophoresis at pH 5.4. Arrows indicate the positions to which authentic sialyloligosaccharides migrated: (a) NeuAc·Gal$_2$·GlcNAc$_2$·Man$_3$·GlcNAc·GlcNAc$_{OT}$; (b)NeuAc$_2$·Gal$_2$·GlcNAc$_2$·Man$_3$·GlcNAc·GlcNAc$_{OT}$; (BPB) bromophenol blue.

Analytical Methods. Radioactivity was determined with a Beckman LS-7000 liquid scintillation spectrometer. Radiochromatoscanning was performed with a Packard Model 7201 radiochromatogram scanner. Radioactivity eluted from Bio-Gel P-4 columns was detected with an Aloka radio liquid chromatograph analyzer RLC-601B. Methylation analysis of oligosaccharides was carried out as described earlier (Endo et al., 1979).

Identification of sialic acids released from Wh λ and Nei λ was performed by trimethylsilylation as follows. Two-hundred micrograms of Wh λ and Nei λ was dissovled in 0.3 mL of 0.1 N HCl and heated at 80 °C for 1 h. The solutions were evaporated to dryness under reduced pressure. After being thoroughly dried in a desiccator over P_2O_5 in vacuo, the samples were trimethylsilylated at room temperature for 1 h and analyzed as described by Yamashita et al. (1983a).

Descending paper chromatography was carried out in the solvent mixture 1-butanol/ethanol/water (4:1:1), on Whatman No. 3MM paper. High-voltage paper electrophoresis was performed in pyridine/acetic acid/water (3:1:387), pH 5.4, at a potential of 73 V/cm for 1.5 h.

Sugar chains were fractionated on columns of Bio-Gel P-4 at 55 °C (Yamashita et al., 1982) with a mixture of glucose oligomers as internal standards to which was added radioactive oligosaccharides [$(5 \sim 10) \times 10^4$ cpm]. Two-milliliter fractions were collected at a flow rate of 0.3 mL/min. Internal standard and radioactive oligosaccharides in column effluents were monitored with a differential refractometer, Shodex RI Model SE-II (Showa Denko Ltd., Tokyo), and with a radio liquid chromatograph analyzer, Model RLC-601B (Aloka Ltd., Tokyo).

Results

Fractionation of Oligosaccharides by Paper Electrophoresis and Gel Permeation Chromatography. Radioactive oligosaccharide fractions obtained from the two BJ proteins were subjected to paper electrophoresis at pH 5.4. As shown in Figure 1, two acidic oligosaccharide fractions were obtained from both samples. The mobilities of the two acidic fractions from Nei λ were identical with two authentic oligosaccharides: NeuAc·Gal$_2$·GlcNAc$_2$·Man$_3$·GlcNAc·GlcNAc$_{OT}$ and NeuAc$_2$·Gal$_2$·GlcNAc$_2$·Man$_3$·GlcNAc·GlcNAc$_{OT}$, respectively (Figure 1B), whereas those from Wh λ were slightly slower (Figure 1A). The molar ratios of fractions 1 and 2 from Wh

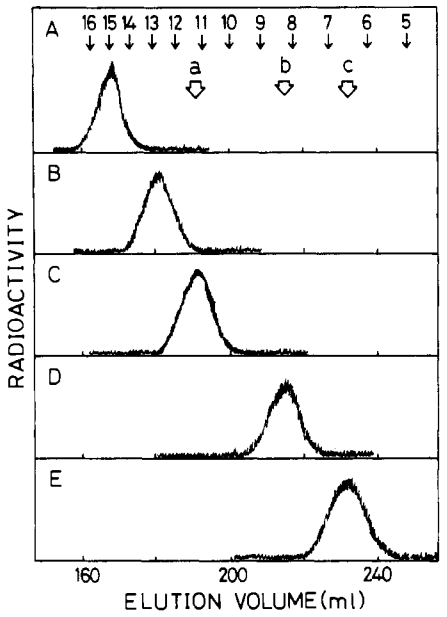


FIGURE 2: Sequential exoglycosidase digestion of fraction 1N obtained from Wh λ. The radioactive sugars were analyzed by Bio-Gel P-4 column chromatography. The solid arrows at the top indicate the elution positions of glucose oligomers (numbers indicate glucose units). The open arrows indicate the elution positions of authentic oligosaccharides: (a) GlcNAc·Man_2·GlcNAc·Man·GlcNAc·Fuc·$GlcNAc_{OT}$; (b) Man_3·GlcNAc·Fuc·$GlcNAc_{OT}$; (c) Man·GlcNAc·Fuc·$GlcNAc_{OT}$. (A) Neutral fraction 1N obtained by sialidase digestion from fraction 1 in Figure 1; (B) radioactive peak in (A) digested with β-galactosidase; (C) radioactive peak in (B) digested with diplococcal β-*N*-acetylhexosaminidase; (D) radioactive peak in (C) digested with jack bean β-*N*-acetylhexosaminidase; (E) radioactive peak in (D) digested with α-mannosidase.

λ and fractions 3 and 4 from Nei λ calculated on the basis of their radioactivities were 32:68 and 30:70, respectively.

When the four acidic oligosaccharide fractions (each containing 1×10^5 cpm) were incubated with 50 milliunits of sialidase in 50 μL of 0.1 M sodium acetate buffer, pH 5.0 at 37 °C for 18 h, they were completely converted to neutral components (data not shown). By shorter sialidase digestion, whereby a part of the original acidic oligosaccharides still remained, fractions 1 and 3 gave only neutral components, while fractions 2 and 4 gave one additional acidic peak with the same mobility as fractions 1 and 3, respectively (data not shown). These results indicated that the oligosaccharides in fractions 1 and 3 contain one sialic acid residue per molecule, and those in fractions 2 and 4 contain two sialic acid residues. All sialic acid residues released from intact Wh λ and Nei λ proteins were identified as *N*-acetylneuraminic acid.

The neutral components obtained from fractions 1–4 by sialidase digestion were named as fractions 1N–4N, respectively. Bio-Gel P-4 (<400 mesh) column chromatography of the four neutral oligosaccharide fractions revealed that the two fractions from the same BJ proteins were the same single oligosaccharide, but the oligosaccharides liberated from the two BJ proteins were different (Figures 2A and 3A).

Sequential Exoglycosidase Digestion of the Neutral Oligosaccharides Obtained from the BJ Proteins. In order to determine the anomeric configuration and the sequence of each monosaccharide in the four neutral oligosaccharides, radioactive fractions 1N–4N were subjected to sequential exoglycosidase digestion, and the reaction product at each step was analyzed by Bio-Gel P-4 column chromatography. Since the same results were obtained from fractions 1N and 2N or fractions 3N and 4N, only the results of fractions 1N and 3N will be documented below.

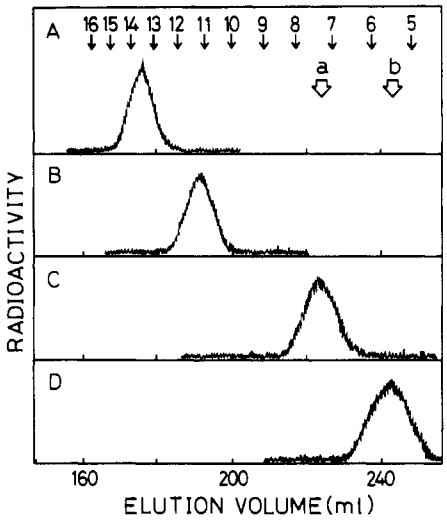


FIGURE 3: Sequential exoglycosidase digestion of fraction 3N obtained from Nei λ. The radioactive sugars were analyzed by Bio-Gel P-4 column chromatography. The solid arrows at the top are the same as in Figure 2, and the open *arrows* indicate the elution positions of authentic oligosaccharides: (a) Man_3·GlcNAc·$GlcNAc_{OT}$; (b) Man·GlcNAc·$GlcNAc_{OT}$. (A) Neutral fraction 3N obtained by sialidase digestion of fraction 3 in Figure 1; (B) radioactive peak in (A) digested with β-galactosidase; (C) radioactive peak in (B) digested with diplococcal β-*N*-acetylhexosaminidase; (D) radioactive peak in (C) digested with α-mannosidase.

When the radioactive 1N was incubated with *diplococcal* β-galactosidase, two galactose residues were removed (Figure 2B). The radioactive product then released an *N*-acetylglucosamine residue by incubation with diplococcal β-*N*-acetylhexosaminidase and was converted to a radioactive oligosaccharide with the same mobility as authentic GlcNAc·Man_2·GlcNAc·Man·GlcNAc·Fuc·$GlcNAc_{OT}$ (Figure 2C). The radioactive oligosaccharide released two additional *N*-acetylglucosamine residues by jack bean β-*N*-acetylhexosaminidase digestion and was converted to a radioactive oligosaccharide with the same mobility as authentic Man_3·GlcNAc·Fuc·$GlcNAc_{OT}$ (Figure 2D). The radioactive oligosaccharide then released two mannose residues on incubation with jack bean α-mannosidase (Figure 2E). The radioactive peak in Figure 2E was shown to have the structure Manβ1→GlcNAcβ1→(Fucα1→)$GlcNAc_{OT}$ by further sequential digestion with β-mannosidase, jack bean β-*N*-acetylhexosaminidase, and *Charonia lampas* α-fucosidase (data not shown).

When radioactive fraction 3N was exposed in turn to diplococcal β-galactosidase and diplococcal β-*N*-acetylhexosaminidase, two galactose residues (Figure 3B) and two *N*-acetylglucosamine residues (Figure 3C) were released, respectively. The radioactive product obtained after the β-*N*-acetylhexosaminidase digestion showed the same mobility as authentic Man_3·GlcNAc·$GlcNAc_{OT}$. The radioactive pentasaccharide released two mannose residues by incubation with jack bean α-mannosidase (Figure 3D). The radioactive peak in Figure 3D was identified as Manβ1→GlcNAcβ1→·$GlcNAc_{OT}$ by sequential digestion with β-mannosidase and jack bean β-*N*-acetylhexosaminidase (data not shown).

From the results of the sequential exoglycosidase digestion described above, the monosaccharide sequences and anomeric configurations of oligosaccharides 1N and 3N can be written as (Galβ1→GlcNAcβ1→Manα1→)$_2$(GlcNAcβ1→)·Manβ1→GlcNAcβ1→(Fucα1→)$GlcNAc_{OT}$ and (Galβ1→·GlcNAcβ1→Manα1→)$_2$Manβ1→GlcNAcβ1→$GlcNAc_{OT}$, respectively.

Methylation Analysis of Oligosaccharides Obtained from

Table I: Methylation Analysis of Fractions A and AN and Mannose Core Obtained from Proteins Wh λ and Nei λ

	molar ratio[a]					
	Wh λ			Nei λ		
	A	AN	core	A	AN	core
galactitol						
2,3,4,6-tetra-*O*-methyl-1,5-di-*O*-acetyl	0.3	2.1	*b*	0.4	2.0	*b*
2,3,4-tri-*O*-methyl-1,5,6-tri-*O*-acetyl	1.9	*b*	*b*	1.7	*b*	*b*
mannitol						
2,3,4,6-tetra-*O*-methyl-1,5-di-*O*-acetyl	*b*	*b*	*2.0*	*b*	*b*	*2.0*
3,4,6-tri-*O*-methyl-1,2,5-tri-*O*-acetyl	*2.0*	*2.0*	*b*	*2.0*	*2.0*	*b*
2,4-di-*O*-methyl-1,3,5,6-tetra-*O*-acetyl	*b*	*b*	1.1	0.9	0.9	1.1
2-mono-*O*-methyl-1,3,4,5,6-penta-*O*-acetyl	0.9	0.9	*b*	*b*	*b*	*b*
fucitol						
2,3,4-tri-*O*-methyl-1,5-di-*O*-acetyl	0.7	0.9	1.0	*b*	*b*	*b*
2-(*N*-methylacetamido)-2-deoxyglucitol						
1,3,5,6-tetra-*O*-methyl-4-mono-*O*-acetyl	*b*	*b*	*b*	0.7	0.8	0.8
1,3,5-tri-*O*-methyl-4,6-di-*O*-acetyl	0.8	0.8	0.9	*b*	*b*	*b*
3,4,6-tri-*O*-methyl-1,5-di-*O*-acetyl	0.9	1.0	*b*	*b*	*b*	*b*
3,6-di-*O*-methyl-1,4,5-tri-*O*-acetyl	3.0	2.8	1.0	2.7	2.9	0.9

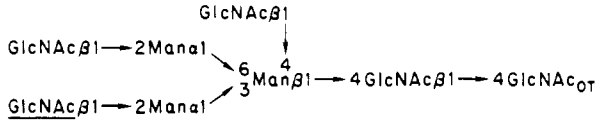
[a] Numbers in the table were calculated by using the italic values as integers. [b] Less than 0.05.

Wh λ and Nei λ Proteins. In order to determine the locations of each glycosidic linkage in the oligosaccharides, deuterium-labeled acidic oligosaccharide mixtures (designated as fraction A) obtained from Wh λ and Nei λ proteins and the neutral fractions (designated as fraction AN) obtained from them by exhaustive sialidase digestion were subjected to methylation analysis (Table I). Data obtained by methylation analysis of the trimannosyl cores of Wh λ and Nei λ proteins (corresponding to the radioactive peaks in Figure 2D and Figure 3C, respectively) are shown in Table I. These data and those from the exoglycosidase digestions indicated that the oligosaccharides from Wh λ and Nei λ contain Manα1→6(Manα1→3)Manβ1→4(Fucα1→6)$GlcNAc_{OH}$ and Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→$4GlcNAc_{OH}$ as their respective core structures.

Comparison of the methylation data of fraction AN and the core from Wh λ indicated that two α-mannosyl residues of the core were substituted at the C-2 position by outer chain moieties. Detection of 1 mol each of 3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-2-deoxyglucitol and 2-mono-*O*-methylmannitol instead of 2,4-di-*O*-methylmannitol in fraction AN indicated that the oligosaccharide in this fraction contains the bisecting *N*-acetylglucosamine residue. Among the 2.8 mol of 3,6-di-*O*-methyl-2-(*N*-methylacetamido)-2-deoxyglucitol, 1 mol should be derived from the core portion. Therefore, the two outer chain moieties of the oligosaccharide should be present as the Galβ1→4GlcNAc group. This conclusion accords with the evidence that all galactose residues were removed by diplococcal β-galactosidase (Figure 2B), which can cleave the Galβ1→4GlcNAc linkage but not the Galβ1→3GlcNAc and Galβ1→6GlcNAc linkages (Paulson et al., 1978).

Comparison of the data obtained by methylation analysis of fractions A and AN indicated that differences were found only in the amounts and kinds of galactitol derivatives. Approximately 1.8 mol less of 2,3,4,6-tetra-*O*-methylgalactitol was detected in fraction A. The decrease of the tetra-*O*-methyl derivative was balanced by the presence of 2,3,4-tri-*O*-methylgalactitol in fraction A. These results indicated that the *N*-acetylneuraminic acid residues of the two acidic oligosaccharides in fraction A are all linked at the C-6 position of the terminal galactose residues.

These findings allow us to propose structures for the sugar chains in Wh λ protein (Figure 4), which are in complete agreement with the results of sequential digestion with diplococcal β-*N*-acetylhexosaminidase (Figure 2C) and jack bean β-*N*-acetylhexosaminidase (Figure 2D). The diplococcal enzyme can release only the underlined β-*N*-acetylglucosamine residue from the following octassacharide (Yamashita et al., 1981):



Comparison of the methylation data obtained from fraction AN and the core from Nei λ protein indicated that the two outer chains, Galβ1→4GlcNAcβ1→, are linked at the C-2 position of the two α-mannosyl residues of the core. The only differences found between fractions A and AN were in the galactose derivatives. Therefore, approximately 40% of the sugar chains in Nei λ protein should be of the monosialyl biantennary variety while the remainder is disialyl biantennary in character (Figure 4).

Discussion

Complete amino acid sequences of the variable regions of the three sugar-containing BJ proteins, WH λ, Nei λ, and Sm λ, are shown in Figure 5. All these glycoproteins belong to subgroup II and have an Asn-X-Ser/Thr sequence in their hypervariable region. Sm λ has a deletion of 81 amino acids in its variable region and contains an N-glycosidically linked sugar chain at asparagine-25 and an O-glycosidically linked one at serine-21 (see Chart I). In contrast, Wh λ and Nei λ contain an N-glycosidically linked sugar chain at asparagine-93 and have no mucin-type sugar chains. In addition, these two BJ proteins are about 84% homologous in their variable regions, indicating that they have probably originated from the same germ line (Kiefer et al., 1980).

Two interesting findings were revealed by comparison of the structures of the sugar chains of the three BJ proteins. The first is that the desialylated carbohydrate moieties of the three Bence Jones proteins are quite homogeneous in contrast to those of whole monoclonal antibodies. Although the data were not given, the oligosaccharide fraction obtained from Sm λ by hydrazinolysis was also separated into two acidic fractions in our laboratory. Both of the neutral oligosaccharide fractions obtained from the two acidic fractions by sialidase digestion gave a single oligosaccharide peak that behaved as 14.5 glucose units on the Bio-Gel P-4 column. That the structure of the oligosaccharide is Galβ1→4GlcNAcβ1→2Manα1→6(Galβ1→4GlcNAcβ1→2Manα1→3)Manβ1→4GlcNAcβ1→

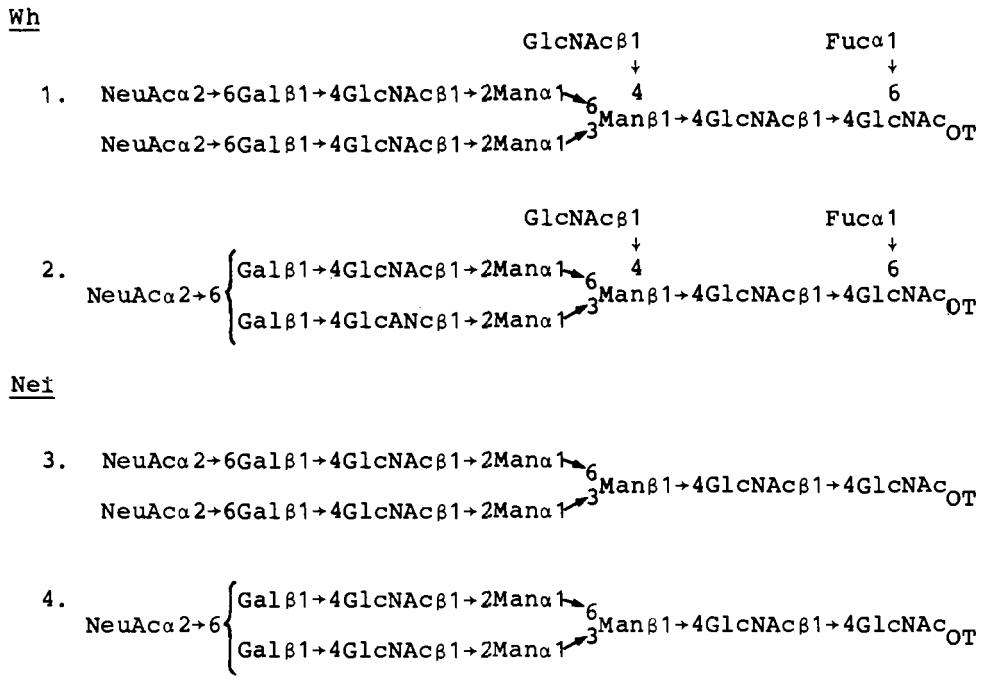


FIGURE 4: Proposed structures of oligosaccharides in fractions 1–4.

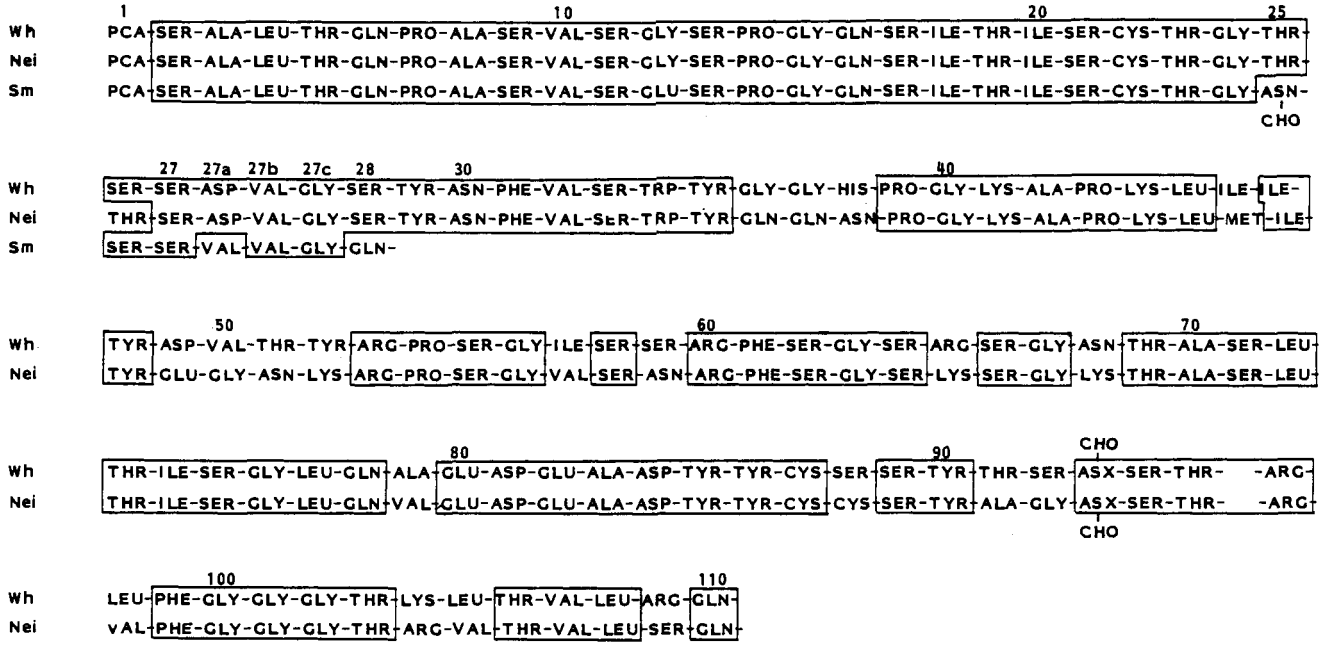


FIGURE 5: Complete amino acid sequence of the variable region of Wh λ, Nei λ, and Sm λ proteins. The data were taken from the reports of Garver & Hilschmann (1972), Garver et al. (1981), and Kiefer et al. (1980).

4(Fucα1→6)GlcNAc$_{OT}$ was confirmed by sequential exoglycosidase digestion in combination with methylation analysis. In their investigations of the sugar chains of 10 IgG myeloma proteins, Mizuochi et al. (1982) found that a variety of oligosaccharide patterns was obtained from these glycoproteins and none of them gave a single sugar chain. They also found that the numbers of asparagine-linked sugar chains in one IgG myeloma protein molecule ranged from two to five according to the protein samples. As suggested by Spiegelberg et al. (1970), a part of the oligosaccharide moieties of myeloma proteins that have more than two sugar chains is probably located in the variable regions of the heavy and/or light chains of these proteins. If the carbohydrate-containing BJ proteins are identical with the light chains of IgG myeloma proteins, the homogeneity found in their carbohydrate moieties is in strict contrast to the microheterogeneity always found in the whole IgG molecule. This may have occurred by structural differences between the light and the heavy chains of the IgG molecule. Since the molecular size of the light chain is small and the glycosylation site is located at the antigen binding area, the site may be more accessible for glycosyltransferases than the hinge region of the heavy chain, which has a more rigid molecular structure. In this context, it might be interesting to study the sugar chains from the variable region of the heavy chains to see if they share the same structure.

The second interesting observation is that the sugar chains of the three BJ proteins are all different. Because of the similarities in the peptide moieties and the glycosylation sites of Wh λ and Nei λ, the structural differences should not be caused by differences in steric effects of their polypeptide moieties. These differences can probably be ascribed to the complement of glycosyltransferases in each myeloma cell,

which may indicate that B cells are a mixture of cells equipped with different sets of such enzymes. Variation in the oligosaccharide patterns of IgG myeloma proteins by Mizuochi et al. (1982) seems to support this possibility. If it is the case, IgG molecules may be classified by their sugar chain structures, although microheterogeneity does exist in the whole IgG molecule. It might be worth investigating whether or not such a subclass of IgG functions differentially.

ACKNOWLEDGMENTS

We are grateful to Professor Fred A. Garver, the Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, GA, for his gift of proteins Wh and Nei. Thanks are also due to Yumiko Kimizuka for her skilled secretarial assistance.

REFERENCES

Abel, C. A., Spiegelberg, H. L., & Grey, H. M. (1968) *Biochemistry 7*, 1271-1278.
Chandrasekaran, E. V., Mendicino, A., Garver, F. A., & Mendicino, J. (1981) *J. Biol. Chem. 256*, 1549-1555.
Ciccimarra, F., Rosen, F. S., Schneeberger, E., & Merler, E. (1976) *J. Clin. Invest. 57*, 1386-1390.
Clamp, J. R., Bernier, G. M., & Putnam, F. W. (1964) *Biochim. Biophys. Acta 86*, 149-155.
Endo, Y., Yamashita, K., Tachibana, Y., Tojo, S., & Kobata, A. (1979) *J. Biochem. (Tokyo) 85*, 669-679.
Garver, F. A., & Hilschmann, N. (1972) *Eur. J. Biochem. 26*, 10-32.
Garver, F. A., Chang, L. S., Kiefer, C. R., Mendicino, J., Chandrasekaran, E. V., Isobe, T., & Osserman, E. F. (1981) *Eur. J. Biochem. 115*, 643-652.
Glasgow, L. R., Paulson, J. C., & Hill, R. L. (1977) *J. Biol. Chem. 252*, 8615-8623.
Grey, A. A., Narashimhan, S., Brisson, J.-R., Schachter, H., & Carver, J. P. (1982) *Can. J. Biochem. 60*, 1123-1131.
Huber, R., Deisenhofer, J., Colman, P. M., & Matsushima, M. (1976) *Nature (London) 264*, 415-420.
Isobe, T., & Osserman, E. F. (1974) *Blood 43*, 505-526.
Kiefer, C. R., Patton, H. M., Jr., Mcguire, B. S., Jr., & Garver, F. A. (1980) *J. Immunol. 124*, 301-306.
Li, Y. T., & Li, S. C. (1972) *Methods Enzymol. 28*, 702-713.
Mizuochi, T., Taniguchi, T., Shimizu, A., & Kobata, A. (1982) *J. Immunol. 129*, 2016-2020.
Nishigaki, M., Muramatsu, T., Kobata, A., & Maeyama, K. (1974) *J. Biochem. (Tokyo) 75*, 509-517.
Nose, M., & Wigzell, H. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 6632-6636.
Paulson, J. C., Prieels, J.-P., Glasgow, L. R., & Hill, R. L. (1978) *J. Biol. Chem. 253*, 5617-5624.
Spiegelberg, H. L., Abel, C. A., Fishkin, B. G., & Grey, H. M. (1970) *Biochemistry 9*, 4217-4223.
Spik, G., Bayard, B., Fournet, B., Strecker, G., Bouquelet, S., & Montreuil, J. (1975) *FEBS Lett. 50*, 296-299.
Sugahara, K., Okumura, T., & Yamashina, I. (1972) *Biochim. Biophys. Acta 268*, 488-496.
Takasaki, S., Mizuochi, T., & Kobata, A. (1982) *Methods Enzymol. 83*, 263-268.
Yamashita, K., Ohkura, T., Yoshima, H., & Kobata, A. (1981) *Biochem. Biophys. Res. Commun. 100*, 226-232.
Yamashita, K., Mizuochi, T., & Kobata, A. (1982) *Methods Enzymol. 83*, 105-126.
Yamashita, K., Hiotoi, A., Matsuda, Y., Tsuji, A., Katunuma, N., & Kobata, A. (1983a) *J. Biol. Chem. 258*, 1098-1107.
Yamashita, K., Hitoi, A., Tsuchida, Y., Nishi, S., & Kobata, A. (1983b) *Cancer Res. 43*, 4691-4695.

Model for Interactions of Amino Acid Side Chains with Watson-Crick Base Pair of Guanine and Cytosine: Crystal Structure of 9-(2-Carbamoylethyl)guanine and 1-Methylcytosine Complex†

Shigeo Fujita, Akio Takenaka, and Yoshio Sasada*

Laboratory of Chemistry for Natural Products, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 227, Japan
Received June 18, 1984

ABSTRACT: As a model of interaction between the guanine–cytosine base pair and carbamoyl group, the crystal structure of 9-(2-carbamoylethyl)guanine–1-methylcytosine complex has been studied by X-ray method. The crystal data are a = 8.540 (1) Å, b = 12.693 (3) Å, c = 14.249 (2) Å, β = 94.02 (1)°, space group $P2_1/c$, Z = 4, d_m = 1.50, d_c = 1.49 g cm^{-3}, and R = 0.10 for 1035 reflections. The bases form a Watson–Crick pair, and the carbamoyl group is hydrogen bonded with O(6) of guanine and N(4) of cytosine in the adjacent pairs. A structural correlation has been found between the hydrogen-bonding pattern and the secondary structural fitting of α-helical segment with B-form DNA.

Recent determination of the three-dimensional structures of DNA binding proteins has furnished a model of their docking or matching with double-stranded DNA (McKay & Steitz, 1981; Anderson et al., 1981; Pabo et al., 1982), but "fine tuning" in the secondary structural fitting is still uncertain. In such mutual recognition, the component–component interaction should play a crucial role, and among several types of the interactions the hydrogen bonds may be the leading part, especialy when high specificity is required.

Detailed information for these interactions would be hardly obtained even if structures of complex crystals between both biomolecules could be solved at low resolution. One approach

† This work was supported in part by Grant-in-Aid for Scientific Research No. 57380026 from the Ministry of Education, Science and Culture, Japan.

0006-2960/85/0424-0508$01.50/0