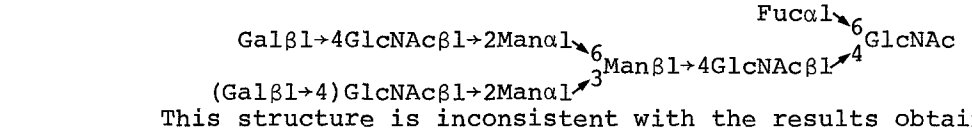
Asparagine-linked Oligosaccharide Chains of IgG: A Revised Structure

by Tadashi Tai, Setsuko Ito, Katsuko Yamashita, Takashi Muramatsu and Akira Kobata

From the Department of Biochemistry
Kobe University School of Medicine
Kusunoki-cho, Ikuta-ku, Kobe, Japan

Received June 16,1975

SUMMARY A complete structure of asparagine linked sugar chains of bovine IgG was determined to be as follows.



This structure is inconsistent with the results obtained from myeloma proteins in that incomplete galactosylation occurred in the branch with α1→3 linked mannosyl residue.

Extensive studies have recently been carried out on structures of asparagine-linked oligosaccharides of myeloma proteins (1-4). Based on various structural features revealed by these studies, Kornfeld and his associates proposed that incomplete galactosylation was restricted in the branch with α1→6 linked mannosyl residue.

In bovine IgG glycopeptides, a part of the glycopeptides was also incompletely galactosylated (5). Treatment of the glycopeptides with β-N-acetylglucosaminidase removed an N-acetylglucosamine residue selectively from the incomplete sugar chain, and the modified glycopeptide became susceptible to endo-β-N-acetylglucosaminidase D (5) and C_I (6). Further removal of the next mannose residue by α-mannosidase digestion made the modified glycopeptide again unsusceptible to the enzymes (5), indicating

that the unsubstituted α-mannosyl residue in the modified glycopeptide is essential for the action of the endoglycosidases. Considering the reported structure of myeloma IgG, this essential mannosyl residue was likely to be located at the C-6 position of the inner β-mannosyl residue. However, by the study with ovalbumin glycopeptides, we found that the essential α-mannosyl residue was located at the C-3 position of the β-mannosyl residue (7). To solve this discrepancy, the study of the complete structure of asparagine linked oligosaccharide from bovine IgG was performed.

MATERIALS AND METHODS

Crude bovine IgG glycopeptides were prepared by pronase digestion of bovine IgG fraction obtained from Sigma Chemical Co. (8). Neutral glycopeptide fraction, comprising 80% of the glycopeptide mixture was isolated by paper electrophoresis at pH 5.4, and was further purified by affinity column chromatography on concanavalin-A Sepharose as described elsewhere (9). About 85% of the glycopeptides were eluted by methyl α-mannoside. This purified glycopeptide fraction was freed from methyl α-mannoside by Sephadex G-25 column chromatography and used for the structural study. The composition of the glycopeptides determined by gas chromatography using ECNSS-M (10) and OV-17 column (11) for sugars and amino acid analyzer for amino acids was as follows (numbers in parentheses indicate molar ratio): mannose (2.9), galactose (1.6), fucose (0.9), glucosamine (3.9), asparatic acid (1.0), serine (0.9) and glycine (0.1).

Endo-β-N-acetylglucosaminidase D from *Diplococcus pneumoniae* (8) and C_I from *Clostridium perfringens* (6) were prepared as described before. The specificity of the two enzymes appeared to be identical (6). Sources of exoglycosidases, and conditions of endoglycosidase and exoglycosidase digestion have been described in detail in previous papers (5,6,8).

Methylation of glycopeptides and oligosaccharides was performed by the method of Hakomori (12). The methylated glycopeptides and oligosaccharide alcohols were subjected to hydrolysis, reduction and acetylation according to Stellner et al (13). Gas chromatographic analysis of methylated sugars by OV-17 column was performed as described elsewhere (14). Standards of methylated sugars were prepared as before (11,14).

N-[^{14}C]Acetylation of glycopeptides (8) and NaB^3H_4 reduction of oligosaccharides (15) were performed as reported. Descending paper chromatography was performed using ethylacetate-pyridine-water (12:5:4) as a solvent.

RESULTS AND DISCUSSION

After sequential removal of galactose and N-acetylglucosamine from non-reducing terminal of sugar chains, all of the purified

glycopeptide fraction was hydrolyzed by endo-β-N-acetylglucosaminidase C_I, releasing two products (II and III in Scheme-1). The structures of the products were determined as indicated in Scheme-1 by the combination of methylation analysis (Table I) and sequential exoglycosidase digestion (6,8).

The linkage between II and III in the original glycopeptide must be GlcNAc→GlcNAc, since reducing end of II was N-acetylglucosamine and fucose occurred as a non-reducing end in intact glycopeptide as judged from its susceptibility to *C. lampas* α-fucosidase. Anomeric configuration of the linkage was concluded to be β, taking the specificity of the endoglycosidase (6) into account. The linkage between the two N-acetylglucosamine residue should be 1→4 since only 3-mono-O-methyl-2-N-methylacetamido-2-deoxyglucitol was detected as a sole mono-O-methyl aminosugar derivative (Table I), and C-6 position of this sugar should be substituted by fucose as judged from the structure of III. Summarizing these results, the core portion of the asparagine linked sugar chains was established as I in Scheme-1.

To this core, 2 moles of β-N-acetylglucosamine and 1.6 moles of β-galactose were linked to form intact glycopeptides. Since β-N-acetylglucosaminidase released 0.4 mole of N-acetylglucosamine from one mole of the glycopeptide fraction (5), we estimated that about 40% of the sugar moiety of the glycopeptide fraction lack one galactose residue linked at N-acetylglucosamine residue in complete chain. Although the intact glycopeptides were resistant to endo-β-N-acetylglucosaminidase D action, 40% of them were hydrolyzed by the endoglycosidase after exhaustive β-N-acetylglucosaminidase digestion (5). This evidence was used for determining the structure of the incomplete sugar chain. The hexasaccharide (IV) released by the endoglycosidase digestion was

Scheme-1.

Stepwise Degradation of Bovine IgG Glycopeptides

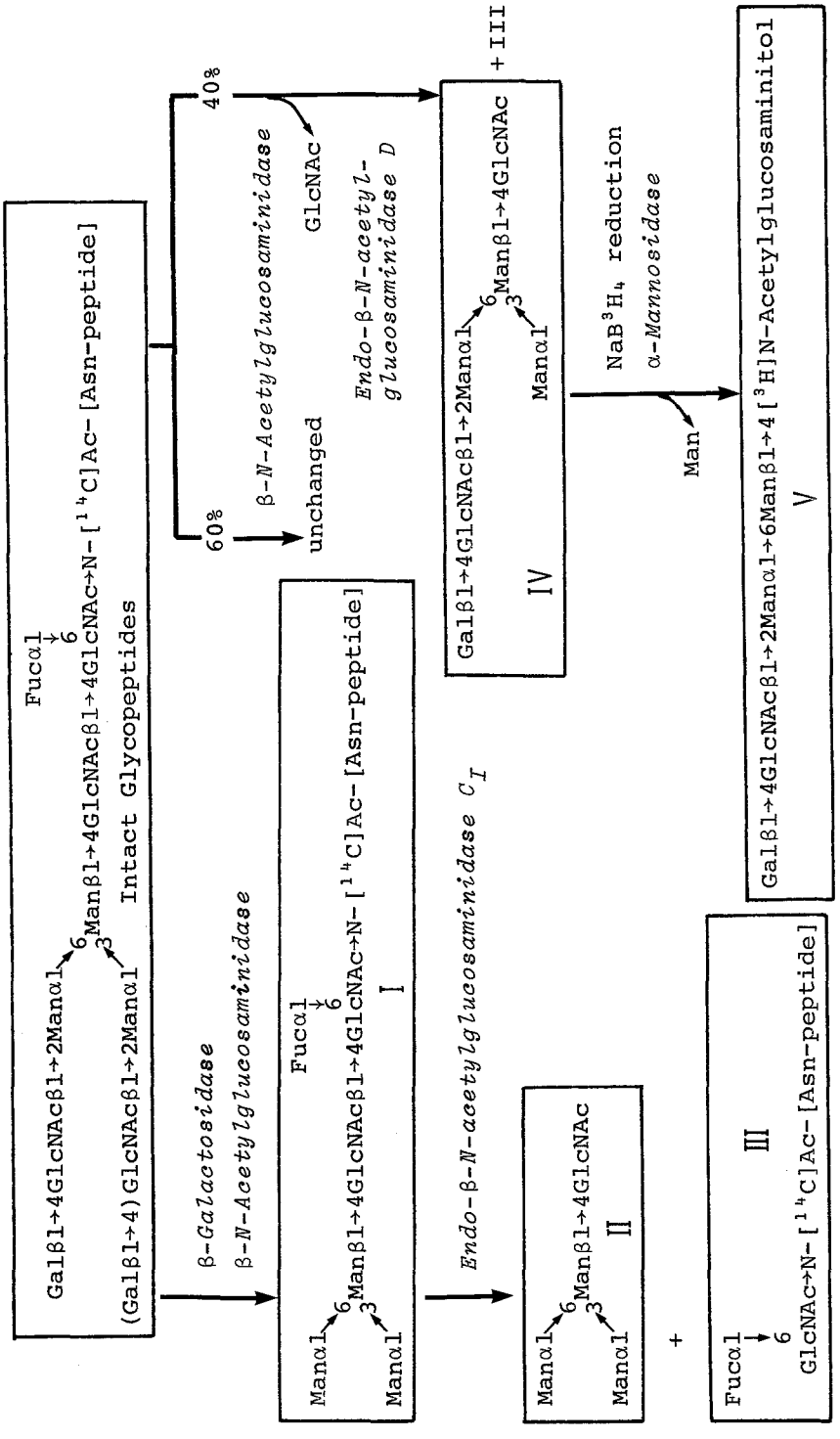


Table I. Methylation analysis of bovine IgG glycopeptides and their fragments

Methylated sugars	Molar ratio*			
	intact	II alcohol	III	V
Mannitol				
2,3,4,6-tetra-O-methyl	-	2.2	-	-
3,4,6-tri-O-methyl	2.0	-	-	0.9
2,3,4-tri-O-methyl	-	-	-	1.0
2,4-di-O-methyl	1.1	1.0	-	-
Galactitol				
2,3,4,6-tetra-O-methyl	1.5	-	-	1.0
Fucitol				
2,3,4-tri-O-methyl	+**	-	+**	-
2-N-methylacetoamido-2-deoxyglucitol***				
1,3,5,6-tetra-O-methyl	-	0.9	-	0.9
3,4,6-tri-O-methyl	0.4	-	-	-
3,4-di-O-methyl	-	-	+****	-
3,6-di-O-methyl	2.7	-	-	0.9
3-mono-O-methyl	0.6****	-	-	-

* Numbers were calculated by making the underlined values as integral numbers.

** Because of interfering substances derived from methylating reagents, quantitative determination of this sugar was not successful although its occurrence was confirmed by mass spectrometry.

*** Identification and quantitative determination of aminosugar derivatives were performed by mass fragmentography as described elsewhere (14).

**** Recoveries of these aminosugars were expected to be low, because they were directly linked to asparagine.

reduced with NaB^3H_4 and the radioactive hexaitol was converted to a pentaitol (V) by α-mannosidase digestion and isolated by paper chromatography (Scheme-1). Methylation analysis (Table I), and sequential exoglycosidase digestion (5) revealed the structure of

the pentaitol as Galβ1→4GlcNAcβ1→2Manα1→6Manβ1→4[^{3}H]N-acetylglucosaminitol. The whole structure of the glycopeptide with incomplete sugar chain can be reconstituted by joining one mole each of α-mannose, the pentaitol (should originally be pentaose), III and β-N-acetylglucosamine together. The former three could easily be combined judging on the basis of the general core structure of the glycopeptides (I in Scheme-1). Methylation analysis showed that among the three mannoses of the glycopeptides, one was 3,6-di-substituted and two were 2-mono-substituted (Table I). Therefore, the remaining N-acetylglucosamine should be linked at the C-2 position of the mannose to form the structure as shown in Fig. 1-A.

The rest of the glycopeptide should contain two β-galactosyl residues and two β-N-acetylglucosamine residues in the outer part. Methylation analysis of intact glycopeptides (Table I) indicates that these sugars must be linked to the core structure at C-2 positions of outer two mannosyl residues. Since galactosyl residues were unsubstituted, and two N-acetylglucosaminyl residues in the outer part were substituted at 4 position, the only possible structure for the rest of the glycopeptide is as shown in Fig. 1-B.

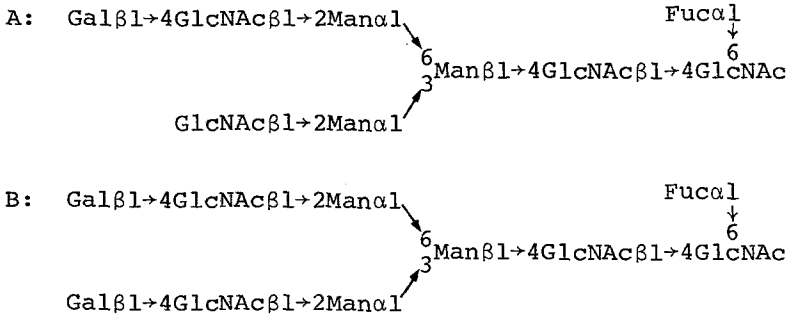


Fig. 1. Structures of asparagine-linked oligosaccharides of bovine IgG

As mentioned above, we have established the whole structure of the asparagine linked oligosaccharides of bovine IgG, and demonstrated that the incomplete galactosylation occurs in the branch with α1→3 linked mannosyl residue. This result is incompatible with the suggestion of Kornfeld and his associates that the incomplete galactosylation occurs in the branch with α1→6 linked mannosyl residue in various myeloma proteins, although it is still possible that the discrepancy comes from the species difference or from the difference of normal and abnormal immunoglobulins.

ACKNOWLEDGEMENTS We thank Miss M. Inohara for her expert secretarial assistance. This work was supported in part by the grant from Ministry of Education of Japan (1974-1975).

REFERENCES

1. Kornfeld, R., Keller, J., Baenziger, J., and Kornfeld, S. (1971) J. Biol. Chem. 246, 3259
2. Hickman, S., Kornfeld, R., Osterland, C. K., and Kornfeld, S. (1972) J. Biol. Chem. 247, 2156
3. Baenziger, J., Kornfeld, S., and Kochwa, S. (1974) J. Biol. Chem. 249, 1897
4. Baenziger, J., and Kornfeld, S. (1974) J. Biol. Chem. 249, 7290
5. Ito, S., Muramatsu, T., and Kobata, A. (1975) Biochem. Biophys. Res. Commun. 63, 938
6. Ito, S., Muramatsu, T., and Kobata, A. (1975) submitted for publication
7. Tai, T., Yamashita, K., Ogata-Arakawa, M., Koide, N., Muramatsu, T., Iwashita, S., Inoue, Y., and Kobata, A. (1975) submitted for publication
8. Koide, N., and Muramatsu, T. (1974) J. Biol. Chem. 249, 4897
9. Ogata, S., Muramatsu, T., and Kobata, A. (1975) J. Biochem. in press
10. Spiro, R. G. (1972) Methods Enzymol. 28, 3
11. Yamashita, K., Tachibana, Y., and Kobata, A. m.s. in preparation
12. Hakomori, S. (1964) J. Biochem. 55, 205
13. Stellner, K., Saito, H., and Hakomori, S. (1973) Arch. Biochem. Biophys. 155, 464
14. Tai, T., Yamashita, K., and Kobata, A. (1975) J. Biochem. in press
15. Takasaki, S., and Kobata, A. (1974) J. Biochem. 76, 783