Structure of Sialylated Fucosyl Lactosaminoglycan Isolated from Human Granulocytes*

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Sialylated fucosyl lactosaminoglycan was isolated from human neutrophilic granulocytes and its structure was elucidated. The lactosaminoglycan glycopeptides were digested by endo-β-galactosidase and "the core portion" and released oligosaccharides were analyzed by permethylation, fast atom bombardment mass spectrometry, and exoglycosidases. In addition, lactosaminoglycan saccharides were obtained by hydrazinolysis and the structures of fractionated sialyl oligosaccharides were analyzed by fast atom bombardment mass spectrometry and permethylation coupled with exoglycosidase treatment. The structure of one of the major components was found to be:

\[
\begin{align*}
\text{Fuc} & \quad \text{1} \\
\text{NeuNAc} & \quad \text{2} \\
\text{Gal} & \quad \text{3} \\
\text{Man} & \quad \text{Man} \\
\text{Fuc} & \quad \text{Fuc} \\
\text{Gal} & \quad \text{Gal} \\
\text{GlcNAc} & \quad \text{GlcNAc} \\
\text{Fuc} & \quad \text{Fuc}
\end{align*}
\]

This structure is unique in that 1) four linear polylactosaminyl side chains are attached to the core portion, 2) the side chain arising from position 4 of 2,4-linked mannose contains one or more α1→3 fucosyl residues, 3) the side chain arising from position 6 of 2,6-linked mannose is terminated with NeuNAcα2→3Gal(Fucα1→3)GlcNAc, sialyl Leα, and 4) the side chain arising from position 2 of 2,4-linked mannose is terminated with sialic acid through α2→6 linkage.

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Polymorphonuclear neutrophils or neutrophilic granulocytes are playing a significant role in nonspecific antibody- and complement-directed host defense. The cell-surface structures of neutrophils are believed to participate in an array of neutrophil functions, such as adherence to vascular endothelium, chemotaxis, ingestion, and microbial killing. In particular, exposure of neutrophils to chemotactic factors induces ruffling of their plasma membrane, increases their surface area, changes their shape from spherical to polarized, decreases their negative net surface charge, and increases their adherence to endothelial cells (1). Decreased neutrophil adherence and chemotaxis have been associated with congenital absence of a membrane glycoprotein in a patient with recur-
rent infections (2). Furthermore, knowledge of the cell-surface antigens of granulocytes is important for transfusion of leukocytes and in the analysis of hematological disorders in the myeloid (granulocyte-monocyte) cell lineage. Despite the importance of granulocyte cell-surface components, systematic studies on membrane glycoproteins of granulocytes have not been made.

We have reported that neutrophilic granulocytes contain a significant amount of neutral lactosaminoglycan with the Galβ1→4(Fucα1→3)GlcNAc structure (3), which is the hapten structure recognized by the majority of recently developed monoclonal antibodies specific to granulocytes (4–8). In this paper, we extend our analysis to the characterization of sialylated lactosaminoglycan isolated from human neutrophil granulocytes which contains the NeuNAcα2→3Galβ1→4(Fucα1→3)GlcNAc terminal structure.

MATERIALS AND METHODS1

RESULTS

Structures of Sialosyl Saccharides Obtained from Gpa—

Based on the results described in the “Miniprint,” the partial structure of sialosyl lactosaminoglycan from human granulocytes can be summarized as follows.

Four poly lactosaminyl side chains are attached to the core portion which has the Manα1→6(Manα1→3)Manβ1→4GlcNAcβ1→4(Fucα1→6)GlcNAc–Asn structure. Some termini of the poly lactosaminyl side chains are sialylated through an α2→6 linkage or an α2→3 linkage to form NeuNAcα2→6Galβ1→4GlcNAc or NeuNAcα2→3Galβ1→4(Fucα1→3)GlcNAc.

In order to more fully characterize the sialylated glycopeptides which appeared to contain a mixture of sialylated saccharide chains, glycopeptide a and glycopeptide b were subjected to hydrazinolysis (9, 19) and saccharides thus obtained were found to be mono-, di-, and trisialylated saccharide based on the determination of sialic acid and hexose content.

Since sialosyl saccharides from Gpb were not available in sufficient amounts, only the saccharides from Gpa were analyzed further.

Structure of A-1—Permethylations analysis indicates that A-1 is composed of 12 lactosaminyl units and contains 1 mol of α2→6-linked sialic acid and 3 mol of nonreducing terminal galactose (Table IV). The same analysis indicates that A-1 contains three fucosyl residues attached to poly lactosaminyl side chains based on the amount of 6-O-methyl-N-acetylgalactosamine. As shown previously, fucose is bound to C-3 of N-acetylgalactosamine to form the Galβ1→4(Fucα1→3)GlcNAc structure (3). Fast atom bombardment mass spectrometry of permethylated A-1 (Fig. 6A) provided a series of signals which are derived from lactosaminyl chains and these are summarized in Table V. These signals apparently are derived from

1 Portions of this paper (“Materials and Methods,” portions of “Results,” Fig. 1–4, and Tables I–III) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 84M-0986, cite the authors, and include a check or money order for $8.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
TABLE V
Compositional assignment for fragment ions derived from saccharides of sialylated fucosyl lactosaminoglycan

| m/z  | Assignment | A-1 | A-2 | A-3 |
|------|------------|-----|-----|-----|
| 1897 | NeuAc·Fuc·Hex·HexNAc⁺ | +   | +   |     |
| 1710 | NeuAc·Hex·HexNAc⁺ | +   |     |     |
| 1536 | Fuc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
| 1362 | Hex·HexNAc⁺ | +   | +   | +   |
| 1274 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1710 | NeuAc·Hex·HexNAc⁺ |     | +   | +   |
| 1448 | NeuAc·Fuc·Hex·HexNAc⁺ | +   |     |     |
possible structures of side chains revealed by fast atom bombardment mass spectrometry

| Number | Structure |
|--------|-----------|
| 1      | Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-O. |
| 2      | NeuNAc-O-Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-O. |
| 3      | NeuNAc-O-Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-O. |
|        | Fuc       |
| 4      | NeuNAc-O-Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-O. |
|        | Fuc       |
| 5      | NeuNAc-O-Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-O. |
|        | Fuc       |
| 6      | Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-O. |
|        | Fuc       |
| 7      | Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-O. |
|        | Fuc       |
| 8      | Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-O. |
|        | Fuc       |
| 9      | Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-O. |
|        | Fuc       |
| 10     | Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-O. |
|        | Fuc       |
| 11     | Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-O. |
|        | Fuc       |
| 12     | Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-Gal-O-GlcNAc-O-O. |

position 2 of 2,4-linked α-mannose, as shown in Fig. 7.

Structure of A-2—Permethylation analysis indicates that A-2 is composed of 12 lactosaminyl units and contains 1 mol each of α2→6-linked sialic acid and α2→3-linked sialic acid and four fucosyl residues attached to polylactosaminyl side chains (Table IV). FAB-MS of permethylated A-2 (Fig. 6B) afforded a series of signals of the same m/z values as those obtained from A-1. Additional signals were present at: m/z 999, 1448, and 1897 which correspond to the fragment ions of composition of NeuNAc-Fuc-Hex-HexNAc, NeuNAc-Fuc-Hex-HexNAc*, and NeuNAc-Fuc-Hex-HexNAc* (Table V). As shown previously (3), sialic acid is bound to galactose and fucose is bound to N-acetylglucosamine. These signals, therefore, suggest the presence of Structure 3, Structure 4, and Structure 5 (Table VI) in the side chain of A-2. Since these fragments can be observed only from sialyl saccharides which were shown by methylation analysis to contain the α2→3 linkage, such as A-2 and A-3 (see Tables IV and V and Fig. 6), it is likely that the sialic acid in this structure is α2→3-linked. This conclusion is supported by the analysis of oligosaccharides released with endo-β-galactosidase treatment as described in the “Miniprint” (see Table III). The same analysis also indicated that Structure 3 is a major component and Structure 4 is a minor one, whereas Structure 5 is absent. These combined results therefore indicate the presence of the following structures:
It is noteworthy that this sialylated fucosyl side chain contains only one fucose since no sialylated chain, which contains two or more fucose residues but no sialic acid, was revealed by FAB-MS (Table V). On the other hand, the side chain, which contains two or more fucose residues but no sialic acid, was revealed by FAB-MS (Table V). Particularly, the presence of m/z 1884 indicates the presence of the following structure (Structure 6 in Table VI) as a component of one of the side chains:

$$\text{Gal}^{\text{a}}\text{Fuc}^{\text{a}}\text{Gal}^{\text{a}}\text{GlcNAc}^{\text{a}}\text{Glc}$$

A portion of this side chain lacks one or two fucosyl residues as judged from the signals at m/z 1710 and 1536. This conclusion is also based on the fact that only one fucosylated side chain without sialic acid is present in A-2 as shown below. Thus, this side chain has various degrees of fucosylation and its structure can be one of Structures 6-12 in Table VI. Since only a limited amount of A-2 was available, it was not determined which structures are actually present among Structures 7-12.

In order to elucidate the distribution of sialic acid and fucose among the four side chains, A-2 was digested with various combinations of exoglycosidases in the same way as A-1. These experiments indicate that the side chain without any substitution arises from position 2 of 2,6-linked α-mannose, and the side chain sialylated through 2,4 linkage and which has no fucose arises from position 2 of 2,4-linked α-mannose. In addition, A-2 was digested extensively with jack bean β-galactosidase, β,N-acetylgalactosaminidase, and almond emulsin α-fucosidase (1-3/4-specific) and this derivative was permethylated. This sample produced 2,3,4-tri-O-methylmannose and 3,4,6-tri-O-methylmannose, indicating that 2-3 linked sialic acid is present in the side chain arising from position 6 of 2,6-linked α-mannose (see Fig. 7).

**Structure of A-2**—Permethylated analysis of A-2 indicates that it consists of 9 lactosaminyl units, two sialic acid residues (2,3- and 2,4-linked), and three fucosyl residues attached to N-acetylgalactosamine in the poly lactosaminyl side chains. FAB-MS afforded a similar series of signals to that observed in A-2 (data not shown). The results indicate that the structure of A-2 is similar to A-2 except that the fucosylated side chain is shorter and contains less fucose. Further analysis of A-2 was not made in the present study due to the limited amount available.

**Structure of A-3**—Permethylated analysis of A-3 indicates that A-3 consists of 8 lactosaminyl units, three sialic acid residues (one 2,4-linked and two of 2,4-linked), and two fucosyl residues in poly lactosaminyl side chains. FAB-MS of permethylated A-3 provided a similar series of fragment ions to that obtained from A-2 (Table V, Fig. 6C). A-3, however, did not produce m/z 1362 for Hex2-HexNAc+ or its fucosylated derivatives nor was m/z 1261 for Fuc2-Hex2-HexNAc detected. These results indicate that the structure of the nonsialylated chain is:

$$\text{Gal}^{\text{a}}\text{Fuc}^{\text{a}}\text{Gal}^{\text{a}}\text{GlcNAc}^{\text{a}}\text{Glc}$$

To elucidate the distribution of sialic acid, A-3 was digested with β-galactosidase, β-N-acetylgalactosaminidase, and α-fucosidase. The product was permethylated and the permethylated sample produced 3,4,6-tri-O-methylmannose with the concomitant loss of 3,6-di-O-methylmannose. Assuming that 2→6-linked sialic acid is present in the same side chain as A-2, the structure of A-3 can be proposed as shown in Fig. 7. A part of the side chains arising from C-2 of 2,6-linked mannose are probably fucosylated since the signal (m/z 999) for NeuNAc:Fuc:Gal:GlcNAc is stronger in A-3 than that in the A-2 sample. In order to confirm this conclusion, A-3 was digested with neuraminidase, β-galactosidase, and β,N-acetylgalactosaminidase. The permethylated sample showed that only a part (0.6 mol) of the chain arising from position 2 of 2,6-linked mannose was removed by this treatment, indicating that 40% of the side chain contains fucose. Since A-3 consists of 8 lactosaminyl units as a total, the total number of lactosaminyl units in three sialylated side chains should be 8. However, a portion of each sialylated side chain contains 3 lactosaminyl units as judged from FAB-MS. These combined results indicate that the number of lactosaminyl units in sialylated side chains varies from 1 to 3 (i.e., m, n, o in Fig. 7 = 0 to 2).

**Fucosyl Lactosaminoglycan Glycoproteins in Granulocytes**—Fig. 8 shows the profile of the granulocyte membrane proteins detected by immunostaining using monoclonal anti-stage-specific embryonic antigen (SSEA-1) antibody. Glycoproteins, which carry lactosaminoglycan with Galβ1→4GlcNAcβ1→Man3, are probably reactive with this antibody (11) and three glycoproteins with molecular weights of 130,000–170,000, 100,000, and 70,000 were revealed. Therefore, these glycoproteins are concluded to be the carriers of fucosyl lactosaminoglycan. Since sialylated fucosyl lactosaminoglycan contains the Galβ1→4(Fucα1→3)GlcNAc terminal structure, it is likely that these glycoproteins also carry the
FIG. 7. Proposed structure of sialylated fucosyl lactosaminoglycan isolated from human granulocytes. A-1, A-2, and A-3 represent the structures of mono-, di-, and trisialosyl saccharide, respectively. On the average, A-1, A-2, and A-3 contain 3, 4, and 2 mol, respectively, of fucose residues in poly-lactosaminyl side chains. m + n + o = 3.0.

This paper describes the structure of sialylated lactosaminoglycan in human granulocytes. The side chains of this lactosaminoglycan are terminated with the following structures: Galβ1→4(Fucα1→3)GlcNacβ1→3, NeuNacα2→3Galβ1→4(Fucα1→3)GlcNacβ1→3, NeuNacα2→6Galβ1→4GlcNacβ1→3 or Galβ1→4GlcNacβ1→3 structure. The NeuNacα2→3Galβ1→4(Fucα1→3)GlcNacβ1→3 structure resides on the poly-lactosaminyl side chain arising from 2,6-linked mannose. The other sialic acid is linked to the poly-lactosaminyl side chain arising from position 2 of 2,4-linked mannose to form the NeuNacα2→6Galβ1→4GlcNac terminal (Fig. 7). The average number of lactosaminyl units is 2 to 3.
3 and four of the polylactosaminyl side chains are bound to α-mannose residues of the core portion, Manα1→3(Manα1→6)Manβ1→4GlcNAcβ1→4(2→Fucα1→3)Galβ1→4GlcNAc→Asn.

Interesting results were obtained by FAB-MS on the length of each side chain. A-2 showed the fragment ions m/z 1710 for Fucα1→2Hexα1→2HexNAcα1→2Hex and 1884 for Fucα1→2Hexα1→2HexNAcα1→2Hex, whereas A-1 lacked the ion of 1884 and A-3 lacked both 1710 and 1884 (Table V, Fig. 6). These differences in fucosylated side chains revealed by FAB-MS correlate well with the results obtained by permethylation analysis. A-2 contains the highest number of fucose residues linked to lactosaminyl units, whereas A-3 contains the smallest number of fucose and total lactosaminyl units (Table IV). These results lead us to conclude that the fragment ions afforded by FAB-MS are reliably indicative of the relative lengths of different side chains present in sialyl saccharides. However, it is not possible at this point to conclude whether the quantitative differences in the intensities of different signals obtained by FAB-MS actually reflect the amount of the structures indicated. This is because we do not know whether the abundance of these ions is affected by their composition. On the other hand, different intensities of the same signal among different samples probably reflect the actual amount of the indicated structure, since the same chemical structures are compared. The use of FAB-MS is indispensable for the analysis of lactosaminoglycan, since it provides sequence information on all side chains of the glycopeptides, which includes the nonreducing terminal portions, and defines the minimum length of long carbohydrate chains.

It is significant that the NeuNAcα2→6Gal terminal is present in the polylactosaminyl side chain arising from position 2 of 2,4-linked α-mannose, whereas the NeuNAcα2→3Gal terminal is mainly present in the polylactosaminyl side chain arising from position 6 of 2,6-linked α-mannose. A similar selective location of sialic acid has also been observed in other glycoproteins (9, 23) although, to our knowledge, the present work is the first report on locations of sialic acid in tetraantennary glycopeptides. Furthermore, fucosylation at N-acetylglucosamine preferentially takes place in polylactosaminyl side chains arising from position 4 of 2,4-linked mannose and position 6 of 2,6-linked mannose (Fig. 7). A similar selective distribution of fucosyl residues in the side chains was reported in the glycopeptide structures of human α1-acid glycoprotein (24) and carcinoembryonic antigen (25), although these glycopeptides mostly contain only 1 lactosaminyl unit in each side chain. It is rather surprising that such preferential sialylation and fucosylation takes place even in long lactosaminyl side chains of granulocyte lactosaminoglycan.

These lactosaminoglycans are carried by glycoproteins of $M_r = 130,000$–$160,000$, $M_r \sim 100,000$, and $M_r \sim 70,000$ (Fig. 8). The glycoprotein(s) of $M_r = 130,000$–$160,000$ is probably the same as Gp 130 reported previously (3). In addition, glycoproteins of $M_r = 135,000$–$170,000$ and $M_r = 105,000$ were reported by Skubitz et al. to carry the Galβ1→4(Fucα1→3)GlcNAcβ1→3 structure (6). Since these workers immunoprecipitated cell surface-labeled proteins, the proteins detected should be expressed on the cell surface. Therefore, these combined results indicate that cell-surface glycoproteins of $M_r = 130,000$–$170,000$ and $M_r \sim 100,000$ carry fucosylated lactosaminoglycan. These glycoproteins might be either rich in the hydrophobic peptide portion or associated with cytoskeletal proteins since the efficient extraction of these glycoproteins was achieved only when 0.2% SDS was included. It is interesting to note that Band 3 glycoprotein, which carries lactosaminoglycan in erythrocytes (9, 10, 26), also associates with cytoskeletal protein, spectrin through ankyrin (27). It has been shown that a glycoprotein carrying lactosaminoglycan gives a characteristic broad band on polyacrylamide gel electrophoresis (9, 26, 28, 29), probably due to the heterogeneity of high-molecular weight carbohydrates. Immune reactive glycoprotein bands shown in the present study also gave broad bands, which is consistent with the property of the glycoproteins carrying lactosaminoglycan. It has been reported that decreased neutrophil adherence and chemotaxis in a patient have been associated with congenital absence of the membrane glycoprotein with $M_r \sim 150,000$ (30). It is of interest to know if this glycoprotein is the same as the fucosyl lactosaminoglycan glycoprotein detected by our studies.

The present studies show that the sialyl lactosaminoglycan of human granulocytes contains a significant amount of NeuNAcα2→3Galβ1→4(Fucα1→3)GlcNAc, sialyl Le" structure. This structure was previously reported in rat brain glycoproteins (31), but not in glycoproteins from human tissue. The isomer of the sialyl Le" structure is NeuNAcα2→3Galβ1→3(Fucα1→4)GlcNAc, sialyl Le" structure, and the presence of sialyl Le" has been reported in human gastrointestinal tumor cells and fetal cells (32). The same structure, however, is absent in normal gastrointestinal cells. Prieels et al. have shown (33) that a fucosyltransferase purified from human milk transfers fucose to both Galβ1→3GlcNAc (type I) and Galβ1→4GlcNAc (type II) structures, which results in the formation of the Galβ1→3(Fucα1→4)GlcNAc (Le") or Galβ1→4(Fucα1→3)GlcNAc (Le") structure. On the other hand, Sheares and Carlson showed that Galβ1→4GlcNAc and Galβ1→3GlcNAc are formed by different galactosyltransferases (34). Therefore, the formation of either Le" or Le" de-
pends on which of the different backbones, type I or type II, is formed by a specific galactosyltransferase. Similarly, an α2→3 sialyltransferase extensively purified from rat liver transfers sialic acid to both Galβ1→4GlcNAc and Galβ1→3GlcNAc (35). However, the α1→3/4 fucosyltransferase mentioned above cannot transfer fucose to NeuAcα2→3Galβ1→3/4GlcNAc (33), nor can the α2→3 sialyltransferase sialic acid to Galβ1→3/4(Fucα1→4)GlcNAc (35). Human granulocytes, gastrointestinal tumors, and fetal tissues must, therefore, have a fucosyltransferase or a sialyltransferase with a broader substrate specificity than the transferases so far purified. It will be interesting to see which of these transferases has the required specificity in these tissues.

Our present and previous studies (3) have yielded detailed structures of granulocyte lactosaminoglycan. We have previously postulated that the amount of lactosaminoglycan parallels the degree of maturation of human myelocytic leukemia cells; immature blastoid cells contain a negligible amount of lactosaminoglycan, whereas promyelocytic leukemic cells express a significant amount of lactosaminoglycan (29). In contrast, Van Beek et al. reported that cancer-related glycopeptides with high-molecular weights are present in leukemic cells at various stages of maturation in order to understand lactosaminoglycan expression in differentiation and leukemogenesis.

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The majority of materials and methods are the same as described in the previous papers:

Antisera and Enzymes: Anti-SSEA-1 monoclonal antibody (31) and anti-NANA monoclonal antibody (121) were kindly donated by Dr. Delr. of the Walter Institute and Dr. Boll of Bernstaud Medical School, respectively. Both monoclonal antibodies recognize anti-SSEA-1 and anti-NANA lectin, respectively. Demethylation was carried out with clostridial neuraminidase (Calbiochem). These results, combined with permethylation studies (Table 3A), indicate the structures of staiyl oligosaccharides shown in Table III. The presence of staiyl oligosaccharides, therefore, is consistent with the following structure shown in Table III.

The neutral oligosaccharides, NANA, hexa-NANA, pentamer, and tetramer, were subjected to fast atom bombardment mass spectrometry (Fig. 8B and C) and, analysis was conducted (Table I). These results suggest that staiyl glycoconjugates resulted from treatment with neutral oligosaccharides, whereas NANA was not digested by permethylation analysis of staiyl glycoconjugates. It is of interest that staiyl glycoconjugates were more resistant to neutral oligosaccharides than to staiyl oligosaccharides (see also the structure of A-3 and reference 9).

In order to elucidate the mechanism of glycosylation in staiyl glycoconjugates, the observation of staiyl oligosaccharides was digested with permethylation analysis of NANA (31). For complete digestion of this glycoconjugate, the glycosylation of relatively low glycosyl residues were further digested with neutral oligosaccharides. As shown in Fig. 9A, staiyl glycoconjugates were identified at the same glycosyl residues produced by treatment with neutral oligosaccharides. These results indicate that staiyl glycoconjugates were more resistant to neutral oligosaccharides than to staiyl oligosaccharides, whereas neutral oligosaccharides were found to be major products from staiyl glycoconjugates. In contrast, staiyl glycoconjugates produced mainly staiyl oligosaccharides and fucose containing NANA, hexa-NANA and pentamer oligosaccharides (Table III).
Glycopeptides were applied to a column (1.0 x 54 cm) of Sephadex G-50 (super fine) equilibrated and eluted with 0.2 M NaCl, each fraction containing 3.7 ml and aliquots (500 uI) were taken for determining radioactivity.

A. Sialyl Gpa was labeled by fast atom bombardment and applied to the Sephadex G-50 column (1.0). The glycopeptides eluted between 48 and 53 after endo-E-galactosidase treatment. The glycopeptides, which were eluted between fractions 48 and 53 after endo-E-galactosidase digestion, were applied to the same column.

B. Sialyl Gpa and sialyl Gpb were labeled by [14C]-Lactosaminoglycan by endo-5-galactosidase. Oligosaccharides released by endo-e-galactosidase were applied to the column.

C. Sialyl Gpa was labeled by 3H5-mannose and applied to the Sephadex G-50 column (1.0). When glycopeptides eluted between fractions 48 and 53 were digested with endo-E-galactosidase and applied to the column of G-50 (1.0).

Fig. 1. Sephadex G-50 gel filtration of glycopeptides obtained from sialyl Gpa and sialyl Gpb.

Glycopeptides were applied to a column (1.0 x 54 cm) of sephadex G-50 (upper fine) equilibrated and eluted with 0.2 M NaCl, each fraction containing 3.7 ml and aliquots (500 ul) were taken for determining radioactivity.

A. Sialyl Gpa was labeled by fast atom bombardment and applied to the Sephadex G-50 column (1.0). The glycopeptides eluted between 48 and 53 after endo-E-galactosidase and applied to the same column.

B. The glycopeptides, which were eluted between fractions 48 and 53 after endo-E-galactosidase digestion, were applied to the same column.

C. Sialyl Gpa was labeled by 3H5-mannose and applied to the Sephadex G-50 column (1.0). When glycopeptides eluted between fractions 48 and 53 were digested with endo-E-galactosidase and applied to the column of G-50 (1.0).
Sialylated Fucosyl Lactosaminoglycan

Table III

| Structure                                      | Sialylated Fucosyl Lactosaminoglycan by Endo-
|                                               | α-Galactosidase |
|-----------------------------------------------|----------------|
| Pentasaccharide                               | Sialylated     |
| Galβ1-3GlcNAcβ1-3Gal                        | Fucosyl       |
| Tetrasaccharide                               | Lactosaminoglyc |