The Glycosylation of Rat Intestinal Muc2 Mucin Varies between Rat Strains and the Small and Large Intestine

A STUDY OF O-LINKED OLIGOSACCHARIDES BY A MASS SPECTROMETRIC APPROACH*

Niclas G. Karlsson‡§, Annkatrin Herrmann‡, Hasse Karlsson‡, Malin E. V. Johansson‡, Ingemar Carlstedt‡, and Gunnar C. Hansson‡

From the ‡Department of Medical Biochemistry, Göteborg University, Medicinaregatan 9A, 413 90 Gothenburg, Sweden and the ¶Department of Cell and Molecular Biology, Section for Molecular Pathogenesis, Lund University, P. O. Box 94, 221 00 Lund, Sweden

The large glycosylated domains obtained from the rat intestinal mucin Muc2 were isolated from the large and small intestine of the inbred rat strains GOT-W and GOT-BW. The expression of the rat Muc2 in the large intestine was confirmed immunohistochemically and by Northern blotting. Released oligosaccharides were structurally characterized by gas chromatography-mass spectrometry (neutral and sialylated species) or by tandem mass spectrometry (sulfated species), and a total of 63 structures was assigned. The large intestinal oligosaccharides were found to be identical between the strains, while the small intestinal glycosylation differed. Until now, detailed structural analysis of oligosaccharides isolated from a single mucin core or mucin domain with different origin have not been performed, and the information of different mucin glycoforms has been limited to immunohistochemistry. Blood group A-determinants (GalNAc1–3(Fucα1–2)Galβ1–, and structures related to the blood group SdCad-related epitope NeuAcαNeuGcα1–3(GalNAcβ1–4)Galβ1–, were found in GOT-BW small intestine, and also in both large intestines. Blood group H-determinants and NeuAcαNeuGcα1–3Galβ1– were found in all samples. Core 1 (Galβ1–3GalNAcα1–), core 2 (Galβ1–3(GlcNAcβ1–6)GalNAcα1–), core 3 (GlcNAcα1–3- GalNAcα1–), and core 4 (GlcNAcβ1–3(GlcαNeuAcβ1–6)GalNAcα1–) were also found in all the samples. The large intestine were enriched in sulfated oligosaccharides and the small intestine contained higher amounts of sialylated species. Sulfation were found exclusively on C-6 of GlcNAc.

The family of highly glycosylated glycoproteins found at the mucosal surfaces are known as mucins (1). Characteristic for mucins is the high degree of O-linked glycosylation known as the mucin-type, where α-N-acetylgalactosamine is linked to serine or threonine of the protein backbone. These amino acids together with proline are mainly found in long and often repeated protein sequences, called mucin domains, that become a scaffold for the glycosylation. The mucins are largely responsible for the physical properties of mucus that serves as a lubricant for the mucosal surface and protects the underlying epithelium from mechanical and chemical stress. However, the identification of several different encoded mucins, and an enormous repertoire of possible mucin oligosaccharides indicates that the tasks for these glycoproteins may be more subtle than the macroscopic properties suggest.

Mucins produced in the intestine are mainly derived from the goblet cells. The major part (at least 80%) of the rat intestinal mucins, measured as protease-resistant mucin domains, can be recovered as an “insoluble” glycoprotein complex in as denaturing conditions as 6.0 M guanidinium chloride (2), providing that shear homogenization is avoided. Two highly glycosylated peptides, named glycopeptide A (gpA) and B (gpB), 650 kDa and 335 kDa, respectively, were isolated by trypsin digestion of subunits from this insoluble mucin complex. A polyclonal antiserum raised against the deglycosylated gpA was used to isolate a cDNA clone (3). This sequence together with two other partial sequences (4–6) have been shown to have homology within separate regions of the intestinal human mucin MUC2 (7), proposing that all three are parts of the rat Muc2 gene. The organization of rat Muc2 gene has recently been further explored and compared with its human homologue (8). The Muc2 mucin has two large domains of sequences rich in serine, threonine, and proline residues, with a small cysteine-rich region in between. It was concluded that the gpB was the smaller domain at the N-terminal side, while gpA was the larger one at the C-terminal side. The two highly glycosylated mucin domains are flanked by less glycosylated cysteine-rich regions. The N- and C-terminal of the human MUC2 and rat Muc2 are believed to be responsible for oligomerization via intramolecular disulfide bridges. Recent biosynthetic studies in the colon cancer cell line LS 174T have shown that a disulfide-stabilized dimer of MUC2 is formed (9), similar to the initial biosynthesis of the von Willebrand factor.

Several reports elucidating the enormous diversity of mucin oligosaccharides do not provide any further information about possible heterogeneity due to the presence of different mucin subpopulations (for example, Refs. 10 and 11). The glycosylation of separate mucins or parts of mucins is rarely described, and the macroscopic properties suggest.

The abbreviations used are: gpA, mucin glycopeptide A; gpB, mucin glycopeptide B; GSL, glycosphingolipid; TBS, Tris-buffered saline; LNF-1, lacto-N-fucopentaose 1 (Fucα1–2Galβ1–3GlcNAcβ1–3Galβ1–4Glc); GC, gas chromatography; MS, mass spectrometry; FAB, fast atom bombardment; CID, collision induced dissociation; Hex, hexose; HexNAc, N-acetylhexosamine; HexNAcβ1, N-acetylhexosaminitol; mAb, monoclonal antibody.
but there is a growing interest due to the fact that the presentation of oligosaccharides will most likely be dependent on the protein backbone (12). We have previously reported a detailed structural characterization of the oligosaccharides from gpA and gpB from an inbred white rat strain (GOT-BW), known to express H-determinants but not A-determinants in the small intestine (2, 8). It was concluded that the glycosylation of the two domains was similar. To further explore the glycosylation of a mucin domain from Muc2, we studied the glycosylation of the small intestinal gpA from a black-and-white inbred rat strain (GOT-BW). This strain has been shown to express blood group A positive glycosphingolipids in the small intestine (13). The analysis was also extended by the identification of oligosaccharides from gpA of rat large intestine of both rat strains, where both strains exhibit blood group A positive glycosphingolipids (14). The question addressed was to what extent a single mucin core exhibit a tissue-dependent glycosylation and if it is influenced by the variation of the glycosylation between individuals. Detailed qualitative and quantitative information from GC-MS, FAB-MS, and MS/MS revealed that the Muc2-derived mucin domain gpA existed as different glycoforms in the small and large intestine. It was further concluded that the small intestinal glycosylation of this domain differed between the two inbred rat strains.

EXPERIMENTAL PROCEDURES

Isolation of Intestinal Mucin Glycopeptide—The mucin gpA was prepared from mucosal scrapings essentially as described (2) from GOT-W and GOT-BW rats small intestines (17 and 9 animals, respectively) and large intestines (7 and 5 animals, respectively). Shortly the procedure involved extracting insoluble material with 95% ethanol, followed by reduction and alkylation with dithiothreitol and nol, followed by reduction and alkylation with dithiothreitol and nicasamidate at 6.0 μg guanidine chloride. The samples were digested with RNase and DNase and two highly glycosylated domains (gpA and gpB) were purified on Sephacryl S-200 and S-500 after tryptic digestion.

Detection of Blood Group Reactivity—GpA (0.1 mg/ml, 50 μl) were coated in soft polystyrene microtiter wells at room temperature overnight, blocked with 2% bovine serum albumin for 1 h, incubated with the antibodies serially diluted (1:2) for 2 h, washed, and detected with 125I-labeled (IODO-GEN, Pierce) anti-mouse antibodies (DAKO, Copenhagen, Denmark) and peroxidase-washing were dried with high radioactivity measured with a γ-counter. The primary antibodies used were anti-blood group H (type 2) (starting dilution 1:25) (DAKO), anti-blood group A (starting dilution 1:100) (DAKO), anti-blood group B (starting dilution 1:50) (DAKO), anti-blood group Lewis b and H type 1 (NS10cl17) (starting dilution 1:50) (The Wistar Institute, Philadelphia, PA), and anti-blood group A type 2 (A005) (starting dilution 1:50) (Memic, Lund, Sweden). The reactivity was measured against glycosphingolipids isolated in our department: A6–1 (A active type 1 hexaglycosylceramide), A6–2 (A active type 2 hexaglycosylceramide), B6–2 (B active type 2 hexaglycosylceramide), H5–1 (H active type 1 pentaglycosylceramide), and H5–2 (H active type 2 pentaglycosylceramide).

Measurement of A-Transferase Enzyme Activity—The large and small intestine were removed and thoroughly washed with phosphate-buffered saline. A 40-cm piece of the small intestine was removed 40 cm from the distal end and mucosal scraping was collected. Mucosal scraping was also collected from the entire large intestine. The samples were homogenized with a Dounce homogenizer (tight piston, 25 strokes) in 35 ml of phosphate-buffered saline and centrifuged at 25,000 × g for 20 min. The pellet was resuspended in phosphate-buffered saline (2 ml for the small intestine and 1 ml for the large) and stored frozen at −20°C. The enzyme assay, 20 μg of LNF-1 (H type 1 pentasaccharide) in 10 μl of cacoaculate buffer, 0.50 n, with 1.5 μl NaCl, pH 6.8, was used as substrate. After the addition of 10 μl of ATP (100 μM), 10 μl of MnCl2 (150 μM) with 100 μl NaN3, and 1.0% Triton X-100, 5 μl of UDP-[14C]GalNAc (0.12 μl), and 65 μl of the reduced pellet, the mixture was incubated at 18°C for 3h and the reaction stopped by heating to 90°C for 30 min. The reaction mixture was desalted on a column with a mixture of AG3-XA and AG 50W-X8 (2 ml) and eluted with 5 ml of water, lyophilized, and redissolved in 100 μl of TBS. An aliquot (25 μl) of the reaction mixture from above was applied to the affinity column at 37°C and fractions of 7 drops (approximately 250 μl) were collected. After the addition of 3 ml of liquid scintillation mixture (Ready Safe, Beckman Instruments, Fullerton, CA) the radioactivity was measured by a Beckman LS6000TA liquid scintillator.

Detection of Rat Muc2 Expression—GpA (0.4 μg) was coated into microtiter plates (Maxisorb, Nalge Nunc, Roskilde, Denmark) by slow evaporation of a water solution at 37°C for 12 h. The plates were further dried in an exciter for 2 h and treated with gaseous hydrogen fluoride in an HF-apparatus (Peptide Institute, Tokyo, Japan) for 18 h and incubated in their original wells by adding 100 μl of phosphate-buffered saline and incubating at 24°C for 37 h. The strips were washed twice with 5 ml Tris-HCl buffer, pH 8.0, containing 0.15 μl NaCl, 0.005% Tween 20, and 0.02% NaN3 and once with 0.15 μl NaCl followed by blocking with 0.1% bovine serum albumin and 6% sorbil in 50 ml Tris-HCl buffer, pH 8.0, with 0.15 μl NaCl, 90 μl CaCl2, 4 μl EDTA, and 0.02 μl NaN3 for 6 h at 37°C. The dissociation enhanced lanthanide fluorimmunoassay was performed as described (16), using a rabbit polyclonal antibody against the deglycosylated gpA from GOT-W small intestine (α-gpA, PH497) (3) or serum from immunized rabbit. As secondary antibody a goat anti-rabbit antiserum (Jackson Immunoresearch, West Grove, PA) labeled with europium was used.

Northern Blot Analysis—RNA (from approximately 50 μg of total RNA) from GOT-W large and small intestine were prepared from mucosal scraping, electrophoresed, blotted, and probed with the VR-1A probe as described (3, 8).

Release and Fractionation of Oligosaccharides—Oligosaccharides from gpA of the various preparations (4–20 mg) were released by β-elimination in 0.05 KOH with 1.0 μl NaBH4 for 45 h at 45°C (1 ml/mg glycopeptide). The reactions were quenched by adding acetic acid, followed by desalting using an AG 50W-X8 column (1.5 ml of resin/ml reaction solution) eluted with water (5 ml/ml resin), and repeated treatment with acetic acid in methanol with subsequent evaporation. Oligosaccharides were applied to DE23 cellulose (Ac- form, 1–2 g) and neutral oligosaccharides were eluted with 50–100 ml of water (containing 1% 1-butanol), followed by elution of the acidic ones with 1.0 ml pyridinium acetate, pH 5.4, and the fractions were lyophilized. An aliquot of the acidic oligosaccharides, or alternatively an aliquot of the total fraction of mucin oligosaccharides (from 2 to 10 mg of glycopeptide), was applied to DEAE-Sephadex A-25 (Ac− form, 0.5 ml of packed resin/mg of glycopeptide). Neutral oligosaccharides (if present) were eluted with methanol (5 ml/ml resin), sialylated oligosaccharides were methanolized (5 ml/ml resin) and permethylated sialylated oligosaccharides were eluted with 1.0 ml pyridinium acetate, pH 5.4. Solvents were removed by rotary evaporation and lyophilization. Sulfated oligosaccharides were desalted by a G-10 column (Pharmacia, Sweden) and redissolved in water containing 5% CH3OH.

Sialylated species (from 2 to 10 mg of gpA) were converted from their methyl esters into N-methylamides by stirring for 10 min in methanol containing 6–12% methylamine.

Monosaccharide and Amino Acid Analysis—Analysis of neutral monosaccharides (1/100–1/20 of either the neutral, sialylated, or sulfated species) were performed after acid hydrolysis in 4.0 M trifluoroacetic acid followed by either conversion into alditol acetates (18), and analysis by GC, or by re-N-acetylation followed by analyses using high performance anion exchange chromatography-pulsed amperometric detection (19). The amino acid composition of the glycopeptides was determined as described (20) with an Alpha Plus amino acid analyzer (Pharmacia).

Characterization of Neutral and Sialylated Oligosaccharides—The neutral oligosaccharides and the N-methylamide derivative of sialylated ones were permethylated with methyl iodide in a slurry of NaOH in dimethyl sulfoxide as described (21, 22). Samples were analyzed by GC and GC-MS after dissolving in ethyl acetate (25–100 μl) and 0.5–1 μl were injected on-column at 70°C. Permethyln,N,N-dimethyl amides of the sialylated species were purified on a Sephadex LH-20 column (7.5 ml) eluted with methanol (0.25 ml/min), before being analyzed by GC and GC-MS. The reaction mixture and the column were prepared as described (17) from fused silica capillaries (11–12 m × 0.25 mm, inner diameter, HT-polymide coated, Chrompack, Milldell, The Netherlands) which were coated with 0.02–0.04 μm of PS284 or SE-54 and cross-linked. Capillary GC was performed on a Hewlett-Packard 5890A gas chromatograph with hydrogen as carrier gas (0.7 bar, linear gas velocity of 114 cm/s at 70°C) including an
Oxygen trap (Oxypurge, Alltech) in the carrier gas line. The flame ionization detector was kept at 390 or 395 °C. Sialylated oligosaccharides were analyzed by a temperature program from 70 °C (1 min) to 200 °C by 50 °C/min and then by 10 °C/min to 390 °C (5 min). Neutral oligosaccharides were analyzed by a linear temperature program from 70 °C (1 min) up to 395 °C (5 min) at 10 °C/min. For GC-MS, helium was used as carrier gas (0.2 bar, linear gas velocity of 75 cm/s at 70 °C) and a Hewlett-Packard 5890A-II gas chromatograph working in a constant flow mode. The gas chromatograph was coupled to a JEOL SX-102A mass spectrometer (JEOL, Tokyo, Japan). The conditions for the mass spectrometer: interface temperature, 385 °C; ion source temperature, 70 °C (1 min) up to 395 °C (5 min); pressure in the ion source region, 5 × 10⁻⁴ pascal.

Characterization of Sulfated Oligosaccharides—Sulfated species were peracetylated with 100–400 µl of pyridine and 50–200 µl of acetic acid anhydride-d₄ for 12 h in room temperature before analyzing the sample with negative ion FAB-MS and MS/MS. Peracetylated sulfated oligosaccharides were dissolved in 50–100 µl of methanol and 1–2 µl was mixed with triethanolamine and ionized by fast atom bombardment for analyzing negative ions. The FAB-MS of the mixture of peracetylated sulfated mucin oligosaccharides was done on MS1 of a JEOL SX/HX 110A four sector tandem mass spectrometer scanning m/z 100–1600; total cycle time, 1.4–1.8 s; resolution, 1400 (m/Δm 10% valley); pressure in the ion source region, 5 × 10⁻⁴ pascal.

RESULTS

Isolation of Glycopeptide A—By reduction and alkylation of mucosa from the large intestine of the GOT-W strain and the small and large intestine of the GOT-BW strain followed by nuclease and trypsin digestion and Sephacryl S-200 and S-500 gel filtration, two highly glycosylated mucin domains (gpA and gpB, respectively) were isolated (Fig. 1). Isolation of the two mucin domains from GOT-W small intestine and their relation to the rat Muc2 mucin have already been reported (2, 8).

Blood group activity of gpA (relative reactivity (%))

| Blood group | Large intestine | Small intestine |
|-------------|-----------------|----------------|
| A           | 110             | 102            |
| B           | 0               | 0              |

Amino acid composition of GOT-W large and small intestinal gpA and Northern blot analysis of rat Muc2 expression in GOT-W large and small intestine. Amino acid composition (A) of GOT-W large intestinal gpA (open boxes) and small intestinal gpA (black boxes). Northern blot (B) from mRNA isolated from GOT-W, the large and small intestine detected with the rat Muc2 probe (VR-1A). kb, kilobase.

So far, rat Muc2 has only been known to be expressed in the rat lung and small intestine (3–6). To verify the observation that Muc2 is present in the large intestine, mRNA was isolated from GOT-W large intestine and Northern blot was performed using the VR-1A clone from rat Muc2 (3, 8) as probe. A single band was detected with identical size (∼12 kilobases) as for Muc2 in the GOT-W small intestine (Fig. 2). The amino acid analysis of GOT-W large intestinal gpA showed a high content of proline, serine, and threonine, with a similar distribution as the GOT-W small intestinal gpA. However, an increased level

FIG. 1. Gel chromatography on Sephacryl S-500 of intestinal mucin glycopeptides obtained after reduction, nuclease and trypsin digestion, followed by chromatography on Sephacryl S-200. The column was eluted with 0.5 M NaAc, pH 7.0, at 40 ml/h. Fractions (15 ml) were collected and analyzed for hexose. The bars indicate the fractions pooled. Separation of GOT-W (a) and GOT-BW (b) small intestinal glycopeptides (A) and large intestinal glycopeptides (B).

FIG. 2. Amino acid composition of GOT-W large and small intestinal gpA and Northern blot analysis of rat Muc2 expression in GOT-W large and small intestine. Amino acid composition (A) of GOT-W large intestinal gpA (open boxes) and small intestinal gpA (black boxes). Northern blot (B) from mRNA isolated from GOT-W, the large and small intestine detected with the rat Muc2 probe (VR-1A). kb, kilobase.
of especially glycine, glutamine/glutamate, and lysine was found. This result together with the slightly lower reactivity of the gpA from large intestine with the α-gpA antiserum (Table I), indicated that there may be additional unidentified biomolecules within these samples. Alternatively, this is a reflection of the variation in glycosylation between the large and small intestine, causing a difference in the size of the tryptic gpA fragments isolated from different sources. The different Muc2 content of the gpA preparations from GOT-W and GOT-BW large intestine, measured as the reactivity with α-gpA antiserum, did not render in different glycosylation (described below). This indicated that glycosylation of the large intestinal gpA as presented here reflected the glycosylation of the rat Muc2 large mucin domain from this tissue, even though small amounts of other glycosylated molecules could be present within the samples.

**Blood Group Reactivity of Glycopeptide A**—From the reactivity with blood group specific antibodies with gpA from the small and large intestine of the GOT-W and GOT-BW rat strains it was concluded that the glycosylation was different both between the tissues and strains (Table I). Blood group A-determinants were found in all of the gpA except for the small intestinal gpA from GOT-W. This correlated with the previously analyzed expression of blood group A active glycosphingolipids (24). To confirm that the presence of blood group A epitopes was due to the action of an GalNAc-α-galactosyltransferase, an in vitro enzyme assay was used. The LNF-1 oligosaccharide was used as substrate and UDP-[14C]GalNAc as sugar nucleotide. After incubation with tissue homogenates the mixtures were analyzed using a blood group A affinity column, where oligosaccharides containing A-determinants are retarded (15). Indeed, the assay specifically excluded the GOT-W small intestine from having A-transferase activity, while the other samples all had an active enzyme, as shown by the retarded signal from [14C]GalNAc-labeled oligosaccharides (Fig. 3).

**Monosaccharide Analyses of Neutral, Sialylated, and Sulfated Oligosaccharides**—The oligosaccharides were released and fractionated into neutral, sialylated, and sulfated species. The number of GalNAcol in each of the neutral (not retarded), sialylated (esterifyable), and sulfated (high salt buffer eluted) fractions is a measure of the number of oligosaccharide chains recovered in each fraction. From Table II it can be concluded that while the distribution within the three oligosaccharide categories from the large intestine was very

---

**TABLE II**

| Fraction | Relative abundance (mol %) |
|----------|---------------------------|
|          | Small intestine | Large intestine |
|          | GOT-W | GOT-BW | GOT-W | GOT-BW |
| Neutral oligosaccharides | 40 | 51 | 53 |
| Sialylated oligosaccharides | 40 | 23 | 19 |
| Sulfated oligosaccharides | 20 | 26 | 28 |

* Data from Carlstedt et al. (2).

* Based on GC measurement of peracetylated alditol acetates measuring the sum of GalNac and GalNAcol. The amount of GalNAcol is then calculated by subtracting the amount of A-determinant containing species from the GC of intact permethylated species assuming that these are the only contributor of GalNAcol to the neutral species.

---

**TABLE III**

| Monosaccharide | Neutral oligosaccharides | Total acidic oligosaccharides | Sialylated oligosaccharides | Sulfated oligosaccharides |
|----------------|----------------------------|-------------------------------|-----------------------------|--------------------------|
|                | GOT-W | GOT-BW | Total | GOT-W | GOT-BW | Total | GOT-W | GOT-BW | Total |
| Small intestine |       |       |       |       |       |       |       |       |       |
| Fuc            | 4.9   |       | 8.0   | 5.4   | 4.7   | 4.9   | 3.3   |       | 0.5   |
| Gal            | 9.9   | 6.5   | 21.0  | 10.2  | 11.6  | 9.2   | 9.4   | 1.0   |
| GlcNac         | 10.6  | 8.0   | 14.0  | 17.3  | 9.7   | 15.6  | 4.3   | 1.7   |
| GalNAc         | 7.6   | 9.3   | 11.0  | 34.0  | 7.0   | 32.4  | 4.0   | 1.6   |
| NeuAc          | NA    | NA    | 4.6   | 1.8   | NA    | NA    | NA    | NA    |
| NeuGe          | NA    | NA    | 4.6   | 2.2   | NA    | NA    | NA    | NA    |
| Total amount   | 37.2  | 30.3  | 63.2  | 70.9  | 33.0  | 62.1  | 21.0  | 4.9   |
| Large intestine |       |       |       |       |       |       |       |       |
| Fuc            | 4.7   | 4.6   | 8.9   | 8.5   | 3.2   | 1.7   | 5.7   | 6.8   |
| Gal            | 6.8   | 6.2   | 26.0  | 19.9  | 10.4  | 6.9   | 15.7  | 13.0  |
| GlcNac         | 3.5   | 4.5   | 19.7  | 21.0  | 6.3   | 7.5   | 13.4  | 13.4  |
| GalNAc         | 7.8   | 8.6   | 16.9  | 18.2  | 5.9   | 5.7   | 11.0  | 12.5  |
| NeuAc          | NA    | NA    | 1.2   | 2.4   | NA    | NA    | NA    | NA    |
| NeuGe          | NA    | NA    | 4.4   | 6.0   | NA    | NA    | NA    | NA    |
| Total amount   | 22.9  | 24.0  | 77.1  | 76.0  | 25.7  | 21.9  | 45.8  | 45.7  |

* NA, not analyzed.

* Data from GOT-W small intestine are from Carlstedt et al. (2).
FIG. 4. Gas chromatograms of permethylated neutral oligosaccharides from gpA of the small and large intestine from rat strains GOT-W and GOT-BW. Oligosaccharides from the GOT-W small (A) and large (B) intestine together with the GOT-BW small (C) and large intestine (D). The neutral oligosaccharides were chromatographed on a 10 m × 0.25-mm column with 0.04-μm stationary phase. On-columns injections at 70 °C (1 min) followed by a linear temperature program of 10 °C/min up to 395 °C (5 min). The numbers refer to structures (Table IV) assigned by GC-MS. Larger peaks identified as non-carbohydrates are marked by an asterisk (*).

TABLE IV

Structures of intestinal neutral oligosaccharides from gpA from the GOT-W and GOT-BW rat strains

| No. | Neutral oligosaccharides | Relative abundance (mole) |
|-----|--------------------------|---------------------------|
|     |                          | Small intestine | Large intestine | Small intestine | Large intestine |
|     |                          | GOT-W | GOT-BW | GOT-W | GOT-BW |
| 1   | GalNAc                   | 4.1   | 3.5   | 1.0   | 1.1   |
| 2.1 | Gal-3GalNAc              | 1.0   | 1.0   | 1.0   | 1.0   |
| 2.2 | GlcNAc-3GalNAc           | 1.0   | 1.0   | 0.5   | 0.2   |
| 3.1 | Fuc-2Gal-3GalNAc         | 2.1   | 0.8   | <0.05 | <0.05 |
| 3.2 | Gal-GlcNAc-3GalNAc       | 1.0   | 0.6   | 0.2   | 0.2   |
| 3.3 | Gal-3GlcNAc-6GalNAc      | 0.7   | 0.2   | <0.05 | <0.05 |
| 3.4 | GlcNAc-Gal-3GalNAc       | 0.1   | 0.1   | 0.1   | 0.1   |
| 3.5 | GlcNAc-3GlcNAc-6GalNAc   | 1.8   | 1.1   | 0.1   | 0.1   |
| 4.1 | Fuc-2Gal-3GlcNAc-3GalNAc | 1.6   | 1.6   | 1.2   | 1.2   |
| 4.2 | Fuc-2Gal-4GlcNAc-3GalNAc | 0.1   | 1.3   | 1.5   | 1.2   |
| 4.3 | GalNAc-Fuc-2Gal-3GalNAc | 2.5   | 1.8   | 0.4   | 0.2   |
| 4.4 | Fuc-2Gal-3GlcNAc-6GalNAc | 0.1   | 0.1   | 0.1   | 0.1   |
| 4.5 | GlcNAc-GlcNAc-Gal-3GalNAc| <0.05 | 0.1   | <0.05 | <0.05 |
| 4.6 | GlcNAc-GlcNAc-GalNAc     | 0.1   | 0.1   | 0.1   | 0.1   |
|     | Structures are base upon mass spectra from GC-MS of permethylated oligosaccharides. HexNAc residues are usually interpreted as GlcNAc based on the monosaccharide composition. GalNAc is believed to be present only as a terminating A-determinant, Hex residues are Gal and deoxyhexoses are Fuc1–2, similar to the linkage in H and A determinants. HexNAcol residues are interpretated as GalNAcol. The interpretation of type 1 chains (Galα1–3GlcNAc) or type 2 chains (Galβ1–4GlcNAc) are based on the relation between fragment ions at m/z 182 and m/z 228, low and high respectively. Substituents on the C-6 branch of the GalNAcol are marked by bold letters.

a The abundances, calculated relative the amount of Gal-3GalNAc, are based on the peak areas in the gas chromatogram divided by the cognate molecular mass.

b Data from Carlstedt et al. (2).
fragmentation of the oxonium ion at m/z HexNAcol. The fragment ion at m/z 883 concluded the branched A-type tetrasaccharide sequence HexNAc-(Fuc-)Hex-HexNAc-3(HexNAc-6)GlcNAc. The fragment ion at m/z 693 was from a further fragmentation of the oxonium ion at m/z 883 by the loss of 1 fucose unit and one additional proton. Empirically this kind of fragmentation has been described to occur only for type 1 chains (Galβ1–3GlcNAcβ1-) and not type 2 chains (Galβ1–4GlcNAcβ1-) (25).

The results from GC and GC-MS imply that the glycosylation of all the gpAs was conducted by a similar initial glycosylation, and that the oligosaccharides became more diversified during the subsequent elongation. The core structures of the oligosaccharides were the same in all the samples (Table IV); core 1 (Galβ1–3GalNAcα1-), core 2 (Galβ1–3(GlcNAcβ1–6)GalNAcα1-), core 3 (GlcNAcβ1–3GalNAcα1-), and core 4 (GlcNAcβ1–3(GlcNAcβ1–6)GalNAcα1-). A family of oligosaccharides (structure 3.4, 4.5, and 6.5, Table IV) was found exclusively in the large intestine. This family contained a series of oligosaccharides with a GlcNAc linked to Gal. GlcNAc was otherwise only found directly linked to the GalNAcol. The length of the oligosaccharides in all samples was estimated using the relative amount of GalNAcol. Overall, the oligosaccharides were relatively short in all four samples, with the average length ranging from 4.7 to 6.1 residues. The same estimations based on the relative amounts given in Table IV would be between 3.1 and 3.7 residues. This discrepancy could be explained by the presence of larger oligosaccharides not analyzable using GC-MS, but may also reflect the presence of peptides with unreleased oligosaccharides attached.

Comparing results from GC and GC-MS (Fig. 4 and Table IV), one can conclude that the large intestinal neutral oligosaccharides were similar, if not identical, between the strains, but differed from the oligosaccharides from the small intestinal gpA. The most obvious difference was that the oligosaccharides from gpA in the large intestine were dominated by blood group A type structure 4.3 (Galβ1–3(Fuc-2)Galβ1–3GlcNAcβ1-) while the small intestinal oligosaccharides, both in GOW and GOW-BW, were dominated by the cognate precursor 3.1 (Fuc-2Galβ1–3GlcNAcβ1-). In general, the blood group A activity, measured as the ratio of any blood group A type structure in Table IV divided by its blood group H type precursor, was higher in the two large intestines, than in the GOW-BW small intestine. The large
intestinal samples were also shown to contain more unidentified contaminants in the gas chromatogram, indicating some impurities from the preparation of these mucin glycopeptides.

**Sialylated Oligosaccharides from Glycopeptide A**—Mass chromatograms of the oxonium fragment ions at m/z 389 [NeuAc]⁺ and at m/z 419 [NeuGc]⁺ are shown in Fig. 6. The fragmentation of the permethylated N,N-dimethylamide derivatives of the sialylated oligosaccharides is similar to that of the neutral ones, with the addition of pronounced fragmentation of the molecular ion due to an α-cleavage of the N,N-dimethylamide side group of the sialic acids, giving an oxonium ion [M-CON(CH₃)]⁺ (26). The fragmentation is exemplified by component 4.2 with the sequence NeuGc-(HexNAc-)Hex-3HexNAcol from GOT-W large intestine (Fig. 7). The [M-CON(CH₃)]⁺ fragment ion at m/z 1088 showed that the oligosaccharide have the composition NeuGc, Hex, HexNAc, HexNAcol, in the ratio 1:1:1:1 and the indicative fragment ions at m/z 260 (terminal HexNAc), m/z 276 (terminal HexNAcol), and m/z 419 (terminal NeuGc), revealed a branched sequence. The fragment ion at m/z 1072 from an α-cleavage between C-4 and C-5 of the GalNAcol concluded the structure.

Sialic acid was found almost exclusively attached to galactose in all samples (Table V), even though gpA of the large intestines and in GOT-BW small intestine also contained disaccharide structures with sialic acid attached directly to C-6 of GalNAcol, indicating the presence of sialyltransferases with additional specificity in these locations.

The monosaccharide composition showed a high content of GalNAc among the sialylated species of gpA from GOT-BW small intestine, indicating the presence of vicinal GalNAc. The structures 4.1, 4.2, 5.3, and 5.4 (Table V), found in GOT-BW but not GOT-W, all had the same general sequence NeuAc/NeuGc-(HexNAc-)Gal-3(R-6)GalNAcol, where R- is the substituent on C-6 of the GalNAcol residue. These sequences are similar to the blood group Sd⁻ and Cad-antigens that are believed to be due to the sugar epitopes NeuAco₂-3(GalNAcβ1-4)Galβ1-4GlcNAcβ1- and NeuAco₂-3(GalNAcβ1-4)Galβ1-3(GalNAcβ1-6)GalNAco₁, respectively. Indeed, the retention time of component 5.3 is identical to that of NeuAco₂-3(GalNAcβ1-4)Galβ1-3(GalNAcβ1-6)GalNAco₁. The terminal HexNAc in structures 4.1, 4.2, 5.3, and 5.4 also found in gpA of the two large intestines was thus assumed to be GalNAc.

**Sulfated Oligosaccharides from Glycopeptide A**—Sulfated oligosaccharides were analyzed as their perdeuterioacetylated derivative by negative FAB-MS, and in the case of high abundant pseudomolecular [M-H]⁻ ions also by MS/MS (Table VI). Sulfated oligosaccharides from the GOT-W small intestinal

![FIG. 7. Mass spectrum from GC-MS of the permethylated sialylated N,N-dimethyl amide (component 4.2 in Table V) from GOT-W large intestine. Positive EI-spectrum of the average of 12 scans from GC-MS (Fig. 6) of the component with a retention time of 14.9 min of a NeuGc containing an Sda/Cad-like epitope at C-3 of the GalNAcol.](image)

**TABLE V**

| No. | Sialylated oligosaccharides* | Relative abundance (mole) |
|-----|-----------------------------|--------------------------|
|     |                             | Small intestine | GOT-W | GOT-BW | GOT-W | GOT-BW |
| 2.1 | NeuAc-6GalNAcol             | 0.1             | 0.1   | 0.2    |
| 2.2 | NeuGe-6GalNAcol             | 0.1             | 0.4   | 0.2    |
| 3.1 | NeuAc-Gal-3GalNAcol         | 1.0             | 1.0   | 1.0    |
| 3.2 | NeuGe-Gal-3GalNAcol         | 0.3             | 0.5   | 1.2    |
| 4.1 | NeuAc-(GalNAc-)Gal-3GalNAcol| 3.4             | 1.1   | 0.3    |
| 4.2 | NeuGe-(GalNAc-)Gal-3GalNAcol| 2.2             | 0.6   | 0.2    |
| 4.3 | NeuAc-Gal-3GlcNAc-6GalNAcol | 0.9             | 1.6   | 0.7    |
| 4.4 | NeuGe-Gal-3GlcNAc-6GalNAcol | 0.6             | 1.4   | 1.0    |
| 5.1 | NeuAc-Gal-3GlcNAc-6GalNAcol | 0.1             |       |        |
| 5.2 | NeuGe-Gal-3GlcNAc-6GalNAcol | 0.1             |       |        |
| 5.3 | NeuAc-(GalNAc-)Gal-3GlcNAc-6GalNAcol| 4.5 | 0.7 | 0.3 |
| 5.4 | NeuGe-(GalNAc-)Gal-3GlcNAc-6GalNAcol| 3.8 | 0.7 | 0.5 |
| 6.1 | NeuAc-Gal-3Fuc-2Gal-GlcNAc-6GalNAcol| 0.2 |       |        |
| 6.2 | NeuGe-Gal-3Fuc-2Gal-GlcNAc-6GalNAcol| 0.2 |       |        |

* Structures are based upon mass spectra from GC-MS of the permethylated N,N-dimethylamidine derivative of sialylated oligosaccharides. Hex residues are interpreted as Gal and deoxyhexoses are Fuc-1–2, similar to the linkage in H-determinants. HexNAc residues are usually interpreted as GlcNAc based on the monosaccharide composition. GalNAc is believed to be present only as NeuAc/NeuGc-(GalNAc-)Gal-, HexNAcol residues are interpreted as GalNAcol. Substituents on the C-6 branch of the GalNAcol are marked by bold letters.

* The abundances, calculated relative the amount of NeuAc-Gal-3GalNAcol, are based on the intensity in the mass chromatogram of the fragment ions at m/z 389 [NeuAc]⁺ and m/z 419 [NeuGc]⁺, respectively.

* Data from Carlstedt et al. (2).
**Glycosylation of Rat Intestinal Muc2**

**Composition and structural assignment of sulfated oligosaccharides from gpA of the two strains GOT-W and GOT-BW**

| [M-H]−a | Sulfated oligosaccharidesb | Relative abundance (mole)c |
|---------|-----------------------------|---------------------------|
|         |                             | Small intestine | Large intestine |
|         |                             | GOT-W | GOT-BW | GOT-W | GOT-BW |
| 1068    | HO,S, HexNAc, HexNAcol     | 0.4   | 0.2   | <0.05 | 0.05  |
| 1072    | GlcNAc-3-HO,S-6GlcNAc-GalNAcol | X     | X     |
|         | HO,S, Hex, HexNAc, HexNAcol| 1.8   | 0.7   | 0.1   | 0.2   |
| 1308    | Fuc-2Gal-3-HO,S-6GlcNAc-GalNAcol | X     | X     | X     |
| 1366    | GlcNAc-Gal1-3-HO,S-6GlcNAc-GalNAcol | X     | X     | X     |
| 1370    | Gal-3-Glucosyl (H2SO4)−6-GlcNAc-GalNAcol | 0.9   | 0.5   | <0.05 | 0.1   |
| 1602    | Fuc-2Gal-GlcNAc-3-HO,S-6GlcNAc-GalNAcol | X     | X     | X     |
| 1606    | GlcNAc-3-Fuc-2Gal-HO,S-6GlcNAc-GalNAcol | X     | X     | X     |
| 1659    | HO,S, Hex, HexNAc, HexNAcol| 0.3   | 0.1   | 0.2   |
| 1663    | Gal-3-Glucosyl (H2SO4)−6-GlcNAc-GalNAcol | X     | X     |
| 1842    | Fuc-2Gal-GlcNAc-3-HO,S-6GlcNAc-GalNAcol | 0.8   | 0.6   | 0.1   | 0.2   |
| 1895    | HO,S, Fuc, Hex, HexNAc, HexNAcol | X     | X     |
| 1899    | HO,S, Fuc, Hex, HexNAc, HexNAcol | 0.6   | 1.0   | 0.1   | 0.1   |
| 1960    | Gal-3-Glucosyl (H2SO4)−6-GlcNAc-GalNAcol | 0.4   | 0.1   |
| 2135    | Fuc-2Gal-GlcNAc-3-Fuc-2Gal-3-HO,S-6GlcNAc-GalNAcol | 0.4   | 0.6   | 0.1   | 0.1   |
| 2196    | Gal-3-Fuc-2Gal-GlcNAc-3-HO,S-6GlcNAc-GalNAcol | X     | X     |
| 2428    | HO,S, Fuc, Hex, HexNAc, HexNAcol | 0.7   | 0.2   |
| 2432    | Gal-3-Glucosyl (H2SO4)−6-GlcNAc-GalNAcol | X     | X     |
| 2498    | HO,S, Fuc, Hex, HexNAc, HexNAcol | 0.2   | 0.1   | 0.1   |

---

a Calculated monoisotopic mass.
b Compositions are based on FAB-MS of perdeuteroacetylated oligosaccharides. Structures found by MS/MS analyses of the corresponding parent ion are marked by X. HexNAc residues are usually interpreted as GlcNAc based on the monosaccharide composition. GalNAc are believed to be present only as a terminanting A-determinant, Hex residues are Gal and deoxyhexoses are Fuc-2, similar to the linkage in H- and A-determinants. HexNAc residues are interpreted as GalNAc. Substituents on the C-6 branch of the GalNAc residue are marked by bold letters.
c The abundances are measured from the intensity of each [M-H]− with cognate isotope signal and calculated relative the [M-H]− ion at \( m/z \) 1602 (HO,S, Fuc, Hex, HexNAc, HexNAcol).
d Data from Karlsson et al. (8).

gpA have already been analyzed in detail by this technique (8) and the results are included as a comparison with the other gpA preparations. In the mass spectra obtained from the MS1 of the tandem mass spectrometer, [M-H]− ions were detected ranging from monosulfated tri- to octasaccharides (Fig. 8). The abundances given in Table VI of the sulfated oligosaccharides were based on the sum of isotope signals from the [M-H]− ions related to each sulfated species. Sequence information of the oligosaccharides was obtained from the more abundant ions when subjected to collision induced dissociation tandem mass spectrometry (CID-MS/MS). The general fragmentation pathways of peracetylated sulfated mucin oligosaccharides detected in the CID-MS/MS spectra have already been described (23) using an extended version of the fragmentation nomenclature of oligosaccharides proposed by Domon and Costello (27). The fragmentation was dominated by intense low mass fragment ions arising from the sulfate group and less intense ions from ring cleavages in the attaching monosaccharide unit (A0 ions). The sequence information was obtained mainly from the \( 1^5X \) ions and (Z-OAc d) ions and the cleavages in the GalNAc residue (A0 cleavages). In Fig. 9 a mixed CID-MS/MS spectrum of the two isomeric pentasaccharides ([M-H]− at \( m/z \) 1602) found in GOT-W large intestine is shown. The C-6 branch of the GalNAc residue was the same of these structures, with a single GlcNAc-6-SO3H residue, while the C-3 branch was the isomeric H-determinant sequence Fuc-2HexNAc-Gal-4(SO3H)−6-GlcNAc-6. The \( 1^5X \)-type fragment ion at \( m/z \) 1349, \( m/z \) 1051, and \( m/z \) 758 together with the \( (Z-OAc)_{d} \)-type fragment ions at \( m/z \) 1242 and \( m/z \) 651 concluded the H-determinant, while the \( 1^5X \)-type fragment ion at \( m/z \) 1349, \( m/z \) 1292, and \( m/z \) 758 together with the \( (Z-OAc)_{d} \)-type fragment ion at \( m/z \) 1242, \( m/z \) 1184, and \( m/z \) 651 concluded the A-determinant.

Similar to the pig small intestine, the rat intestinal oligosaccharide sulfation was mainly directed to C-6 of the GlcNAc residue adjacent to the GalNAc residue (23). A C-6 sulfation is also concluded from CID-MS/MS spectra of less intense [M-H]− ions, even though no complete sequence information could be obtained. The position of the sulfate group could be deduced from the presence of the fragment ion at \( m/z \) 139 (CHO-CHO-\( \text{SO}_3 \)) typical for C-6 sulfation, and the absence of
fragment ions arising from C-3 and C-4 sulfation (result not shown).

DISCUSSION

Previous investigations have established that rat Muc2 is expressed in both the small intestine and in the lungs (4–6). Here, we presented evidence that this mucin is also present in the large intestine, using both immunochemical detection and identification of an mRNA transcript with the same size as rat small intestinal Muc2. The human MUC2 homologue is also expressed both in the large and small intestine (28). The slightly lower Muc2 reactivity of gpA from the large intestine (Table I) and the differences in amino acid composition (Fig. 2) suggested that there could be other unidentified material present in these preparations. In addition to the large mucin glycopeptide (gpA) in rat Muc2 the glycosylation of the smaller glycopeptide (gpB) (Fig. 1) has also been analyzed. The glycosylation of gpA and gpB from the small intestine of the GOT-W strain has been shown to be almost identical (2). This was also observed for the glycosylation of the corresponding gpA and gpB from the large intestine of the GOT-W strain (2). This suggests that the contaminations observed in gpA from the large intestine was not affecting the results.

The glycosylation of the large mucin domain of the Muc2 in the rat intestine was demonstrated to be different in the large and small intestine, but also that there is a variation of the glycosylation in the small intestine between rat strains. Previous investigations of rat intestinal mucin oligosaccharides have revealed that both core 1, core 2, core 3, and core 4 oligosaccharides are present (2, 29). These core structures were also found here. However, we found no obvious differences in the abundance of these core structures in the two rat strains and between the small and large intestine, indicating a similar regulation of the initial oligosaccharide biosynthesis.

The expression of blood group A-determinants in the GOT-W and GOT-BW strain large intestine as well as in the GOT-BW but not in GOT-W small intestine, have previously been reported for the glycosphingolipids (24). Here, the oligosaccharides from the large glycosylated tandem repeat of rat Muc2 mucin demonstrated the same feature shown both by GC-MS (Table II), and by using blood group A-directed monoclonal antibodies (Table I). The findings of blood group A-determinant containing structures in GOT-BW small intestine, and GOT-BW and GOT-W large intestine correlated with the measured blood group A enzyme activity in homogenates from these tissues.

Among the sialylated oligosaccharides a family of structures (NeuAc\(\text{a}_{2}–3\text{GalNAc}_{b}\)) has been isolated from erythrocytes of Cad-positive individuals (30) and is believed to be involved in the rare Cad-blood group antigen, while the more common Sd\(^{b}\)-blood group (92% of Caucasian population are positive (31)), is shown
to be expressed on both glycosphingolipids and glycoproteins. The Sd\(^+\)-active structures share the trisaccharide epitope with the Gal\(\alpha_1\)-glycosphingolipid and Sd\(^+\)C3d-active structures, where Gal\(\alpha_1\)- has been shown to with-

| Blood group type | Epitope | Small intestine | Large intestine |
|------------------|---------|-----------------|-----------------|
| H type 1         | Fuc\(\alpha_1\)-2Gal\(\beta_1\)-3GlcNAc | + \(a\) + \(a\) + \(a\) | + \(a\) + \(a\) + \(a\) |
| H type 2         | Fuc\(\alpha_1\)-2Gal\(\beta_1\)-4GlcNAc | + + + | + + + |
| A type 1         | GalNAc\(\alpha_1\)-3\(\alpha\)-Fuc\(\alpha_1\)-2Gal\(\beta_1\)-3GlcNAc | + + + | + + + |
| A type 2         | GalNAc\(\alpha_1\)-3\(\alpha\)-Fuc\(\alpha_1\)-2Gal\(\beta_1\)-4GlcNAc | + + + | + + + |
| ALex\(a\)        | GalNAc\(\alpha_1\)-3\(\alpha\)-Fuc\(\alpha_1\)-2Gal\(\beta_1\)-4GlcNAc | + + + | + + + |
| BLe\(a\)         | Gal\(\alpha_1\)-3\(\alpha\)-Fuc\(\alpha_1\)-2Gal\(\beta_1\)-4Fuc\(\alpha_1\)-3GlcNAc | + + + | + + + |

From Breimer et al. (33).

From Hansson (14).

From Hansson (13).

From Ångström et al. (34).

REFERENCES

1. Gendler, S. J., and Spicer, A. P. (1995) Annu. Rev. Physiol. 57, 607–634
2. Carlstedt, I., Herrmann, A., Karlsson, H., Shoham, J., Fransson, L.-A., and Hansson, G. C. (1993) J. Biol. Chem. 268, 18771–18781
3. Hansson, G. C., Baeckström, D., Carlstedt, I., and Klinga-Levan, K. (1994) Biochem. Biophys. Res. Commun. 198, 181–190
4. Xu, G., Huan, L., Khatri, I. A., Wang, D., Bennett, A., Fahim, R. E. F., Forstner, G. G., and Forstner, J. F. (1992) J. Biol. Chem. 267, 5401–5407
5. Xu, G., Khatri, I. A., Sajjan, U. S., McCool, D., Wang, D., Jones, C., Forstner, G., and Forstner, J. F. (1992) Biochem. Biophys. Res. Commun. 183, 821–828
6. Ohmoori, H., Dohman, A. F., Gallop, M., Teada, T., Kai, H., Gom, J. R., Kim, Y. S., and Basabash, C. B. (1994) J. Biol. Chem. 269, 17833–17840
7. Gom, J. R., Hicks, J. W., Toribara, N. W., Siddiki, B., and Kim, Y. S. (1994) J. Biol. Chem. 269, 2440–2446
8. Karlsson, N. G., Johansson, M. E. V., Askar, N., Karlsson, H., Gendler, S. J., Carlstedt, I., and Hansson, G. C. (1996) Glycoconjug. J. 13, 823–831
9. Asker, N., Baeckström, D., Axelsson, M. A. B., Carlstedt, I., and Hansson, G. C. (1999) Biochim. Biophys. Acta 140–143
10. Klein, A., Carnoy, C., Lamblin, G., Roussel, P., vanKuik, J. A., Vliegenthart, R. A. (1988) Carbohydr. Res. 211, 207–217
11. Larson, G., Karlsson, H., Hansson, G. C., and Pimlott, W. (1987) Biochim. Biophys. Acta 892, 291–292
12. Dakour, J., Lindblad, A., and Zoph, D. (1987) Anal. Biochem. 161, 140–143
13. Baeckström, D., Hansson, G. C., Nilsson, O., Johansson, C., Gendler, S., and Lindblad, L. (1991) J. Biol. Chem. 266, 21557–21569
14. Karlsson, N. G., Karlsson, H., and Hansson, G. C. (1995) Glycoconjug. J. 12, 69–76
15. Yang, H., and Hakomori, S. (1968) J. Biol. Chem. 246, 171–179
16. Karlsson, N. G., and Hansson, G. C. (1995) Anal. Biochem. 224, 538–541
17. Spackman, D. H., Stein, W. H., and Moore, S. (1958) Anal. Chem. 30, 1190–1206
18. Cucchin, I., and Kerek, F. (1984) Carbohydr. Res. 131, 207–217
19. Larson, G., Karlsson, H., Hansson, G. C., and Pimlott, W. (1987) Carbohydr. Res. 161, 1440–1447
20. Karlsson, N. G., Karlsson, H., and Hansson, G. C. (1996) J. Mass Spectrom. 31, 560–572
21. Hansson, G. C. (1988) in The Molecular Immunology of Complex Carbohydrates (Wu, A. M., ed) pp. 465–494, Plenum Press
22. Smith, E. L., Kibbinn, M., Karlsson, K.-A., Pascher, I., and Samuelsson, B. E. (1975) Biochim. Biophys. Acta 388, 171–179
23. Karlsson, N. G., Karlsson, H., and Hansson, G. C. (1995) Blood Cell Biochemistry (Cartron, J.-P., and Rouger, P., eds) Vol. 6, pp. 351–375, Plenum Press, New York
24. Sob, P. C. S., Donald, A. S. R., Feeny, J., Morgan, W. T. J., and Watkins, W. M. (1989) Glycoconjug. J. 6, 319–332
25. Breimer, M. E., Falk, K.-E., Hansson, G. C., and Karlsson, K.-A. (1982) J. Biol. Chem. 257, 50–59
26. Ångström, J., Falk, P., Hansson, G. C., Holgersson, J., Karlsson, K.-A., Strömberg, N., and Thunberg, J. (1987) Biochim. Biophys. Acta 926, 79–86
27. Smith, A. C., and Podolsky, D. K. (1989) Clin. Gastroenterol. 15, 815–837
28. Corfield, A. P., Williams, A. J. K., Clamp, J. R., Wagner, S. A., and Mousand, R. A. (1988) Clin. Sci. 74, 71–78
29. Corfield, A. P., Wagner, S. A., O'Donnell, L. J. D., Durdy, P., Mousand, R. A., and Clamp, J. R. (1993) Glycoconj. J. 10, 72–81