Gangliosides of Human Kidney*

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Five gangliosides isolated from human kidney have been characterized. The two main fractions were shown to be typical extraneural gangliosides in having lactose as their neutral carbohydrate moiety. Their structures were identified as: AcNeu(a2-3)Gal(b1-4)Glc(b1-1)Cer and AcNeu(a2-3)Gal(b1-4)Glc(b1-1)Cer. The two main hexosamine-containing gangliosides are structurally related to human blood group substances of glycosphingolipid nature. The following structures are postulated: AcNeu(a2-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer and AcNeu(a2-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc(b1-1)Cer. The third hexosamine-containing ganglioside belongs to a different series of glycolipids and was shown to have the structure of a major ganglioside of human brain: AcNeu(a2-3)Gal(b1-3)GalNAc(b1-4)(AcNeu(a2-3))Gal(b1-4)Glc(b1-1)Cer. The fatty acid structure of different gangliosides was shown to resemble that of neutral glycolipids of human kidney with the nonhydroxy acids C18:0, C20:0, and C22:0 as major components.

Gangliosides are neuraminic acid-containing glycosphingolipids. They are constituents of cellular membranes and are localized mainly in the surface membrane (1-3). Gangliosides can function as antigens and mediators of contact recognition between cells (4, 5).

During the past few years, considerable work has been carried out to elucidate the structures of the fucose-containing neutral glycosphingolipids which show blood group activity (6, 7). The structures of brain gangliosides have been well established (8). Gangliosides containing lactose as their neutral carbohydrate moiety are known to predominate in extraneural tissues (9-13), but the studies on more complex fractions are mainly restricted to sialoligosaccharides released from ganglioside preparations (12-14). Recently, a simplified method for isolation of gangliosides as intact molecules was developed (12-14). Recently, an improved method for isolation of gangliosides as intact molecules was developed (8). Gangliosides containing lactose as their neutral carbohydrate moiety are known to predominate in extraneural tissues (9-13), but the studies on more complex fractions are mainly restricted to sialoligosaccharides released from ganglioside preparations (12-14).

The methods of analysis are described in the adjacent supplement.*

**EXPERIMENTAL PROCEDURES**

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* This investigation was supported by the Sigrid Juslius Foundation and by the National Research Council for Medical Sciences, Finland.

* Details of the methylation and characterization of gangliosides I to V are presented in a miniprint format immediately following this paper. (Figs. 3 and 4 and Tables I and II will be found on p. 7520.) For the convenience of those who prefer to obtain this in the form of 10 pages of full size photocopies, it is available as JBC Document Number 76M-476. Orders should specify the title, authors, and reference to this paper, the JBC Document Number, and the number of copies desired. Orders should be addressed to The Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Maryland 20014, and must be accompanied by a remittance to the order of the Journal in the amount of $1.50 per set of photocopies.

RESULTS

The results of structural studies are summarized in Fig. 1. The main gangliosides of human kidney (I and IV) contain lactose as their neutral carbohydrate moiety. The tetrasaccharide core of gangliosides II and III is lacto-N-neotetraose, and that of ganglioside V is ganglio-N-tetraose.

The bonding positions of hexoses and hexosamines were determined by methylation (see the supplement†). In all gangliosides studied, neuraminic acid was susceptible to the action of an end group hydrolase, neuraminidase. Vibrio cholerae neuraminidase hydrolyzed the neuraminic acid of gangliosides I to IV and about half of the neuraminic acid from ganglioside V. The neuraminic acid of ganglioside V was, however, totally hydrolyzed by Clostridium perfringens neuraminidase in the presence of sodium cholate. The treatment with C. perfringens neuraminidase in the presence of cholate is expected to cleave the neuraminic acid near the lipid moiety of ganglioside (18, 19). In order to study the possible substitution of neuraminic acid on C-8, periodate oxidation was carried out. Neuraminic acid was completely oxidized in all gangliosides but in IV, in which 49% of neuraminic acid was resistant. Therefore, neuraminic acid occurs only at the terminal position in gangliosides I, II, III, and V. In ganglioside IV, one of the two neuraminic acid units is terminal, the other is substituted on C-8 (Fig. 1). The recoveries of the hexose and hexosamine residues after periodate oxidation were in accordance with the analysis of glycosidic linkages by methylation.

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Methylation analysis and periodate oxidation do not provide information on sugar sequence except for the terminal sugar at the nonreducing end of the oligosaccharide chain. Therefore, partial acid hydrolysis of gangliosides was carried out. The sequence of monosaccharides was concluded from the analysis of tetra-, tri-, di-, and monoglycosylceramides by thin layer chromatography and sugars released by gas-liquid chromatography (see the supplement). Partial acid hydrolysis also gives information on ring sizes of sugars. Furanosidic linkages are known to be cleaved much faster than pyranosidic linkages (20-22). The terminal galactose residue has a pyranose structure in all gangliosides on the basis of methylation analysis after neuraminidase treatment (23). The terminal sugar in the neutral glycolipid was identified by methylation analysis after neuraminidase treatment. The anomeric nature of the component hexoses and hexosamines was studied by partial acid hydrolysis and by methylation (the terminal sugar in the neutral glycolipid was identified by methylation analysis after neuraminidase treatment). The anomeric nature of the component hexoses and hexosamines was confirmed. In order to study the sequence and anomeric conformation of sugar residues, enzymic hydrolysis is commonly used. In the present work, the sequence of monosaccharides was studied by partial acid hydrolysis and by methylation (the terminal sugar in the neutral glycolipid was identified by methylation analysis after neuraminidase treatment). The anomeric nature of the component hexoses and hexosamines was studied by CrO₃ oxidation in acetic acid. The method used for analysis of sequence and anomeric conformation obviates some difficulties characteristic of enzymic hydrolysis. For instance, α- and β-glycosidases are difficult to obtain in pure form, and they may have other structural requirements regarding the substrate than the type of monosaccharide and the glycosidic linkage to be hydrolyzed. When working with lipids, the limited solubility of substrate can cause incomplete hydrolysis. On the other hand, the use of CrO₃ oxidation for anomeric study has also its restrictions. Furanosidic sugars and the monosaccharides having free hydroxyl groups (acetylated in the present work) are readily oxidized.

The studies on the anomeric nature of glycosidic linkages of gangliosides have been mainly restricted to brain gangliosides. A β configuration of the glycosidic bonds in brain gangliosides has been suggested on the basis of the Hudson rule (27). The problem was recently studied with purified α- and β-glycosidases (28). The β stereoconfiguration of the terminal galactose and the penultimate N-acetylgalactosamine residues in GM₃ from ox brain [Gal-GalNAc-(AcNeu)-Gal-Glc-Cer] was confirmed by stereospecific glycosidase treatments. It was also shown that the bulk of the internal galactose residue and at least 15% of the lipid-bound glucose residue are β glycosidically linked with a small percentage of α-linkages in these monosaccharides (29). Incomplete hydrolysis of the internal monosaccharide residues of the glycolipid could have been due to the limited solubility of the substrate. The present work demonstrates that the bulk of all monosaccharides (except neuraminic acid and fucose) are β-linked in the mixture of human brain gangliosides, in the Tay-Sachs ganglioside (GM₁) of human brain, in the mixture of pig brain gangliosides, in the main ganglioside of pig brain (GM₁), in the two hematosides of bovine kidney, and in the five fractions of human kidney gangliosides (Table II). The results of CrO₃ oxidation are not sufficiently clear as to allow the detection of a few per cent of α-linkages.

It is established in the present work that the main gangliosides of human kidney have the structures of N-acetylatedi-aminylactosylceramide (ganglioside I) and of di-N-acetylatedi-aminylactosylceramide (ganglioside IV). The two main hexosamine-containing gangliosides of human kidney, ganglio-

Fig. 1 Suggested structures for gangliosides of human kidney.

I Gal(β1-4)Glc(β1-1)Cer αAcNeu
II Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1)Cer αAcNeu
III Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1)Cer αAcNeu
IV Gal(β1-4)Glc(β1-1)Cer αAcNeu
V Gal(β1-3)GalNAc(β1-4)Gal(β1-1)Cer αAcNeu

The analysis of fatty acids by gas-liquid chromatography

mass spectrometry (26) revealed C₁₈₀₆, C₁₈₀₇, and C₂₆₈₂₆ as major components in all gangliosides. All gangliosides but V contained also α-hydroxy fatty acids (for more detailed results see the supplement).

DISCUSSION

The technique of permethylation can be used to study the substitution sites on hexoses and hexosamines of gangliosides. Methylation analysis by gas-liquid chromatography-mass spectrometry was recently applied also for neuraminic acid residues (as methyl glycoside methyl esters), and the present results concerning the substitution on neuraminic acid were confirmed. In order to study the sequence and anomeric conformation of sugar residues, enzymic hydrolysis is commonly used. In the present work, the sequence of monosaccharides was studied by partial acid hydrolysis and by methylation (the terminal sugar in the neutral glycolipid was identified by methylation analysis after neuraminidase treatment). The anomeric nature of the component hexoses and hexosamines was studied by CrO₃ oxidation in acetic acid. The method used for analysis of sequence and anomeric conformation obviates some difficulties characteristic of enzymic hydrolysis. For instance, α- and β-glycosidases are difficult to obtain in pure form, and they may have other structural requirements regarding the substrate than the type of monosaccharide and the glycosidic linkage to be hydrolyzed. When working with lipids, the limited solubility of substrate can cause incomplete hydrolysis. On the other hand, the use of CrO₃ oxidation for anomeric study has also its restrictions. Furanosidic sugars and the monosaccharides having free hydroxyl groups (acetylated in the present work) are readily oxidized.

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3 The abbreviations of Svennerholm (17) are used for gangliosides.
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The present results suggest that ganglioside II has the structure of N-acetylenuraminilacto-N-neotetraosylceramide. A similar structure has been recently proposed for several human extraneural gangliosides (12, 14, 29-33). A new structure of N-acetylneuraminyllacto-N-neotetraosylceramide. A similar structure has been recently proposed for several human kidney gangliosides and neutral glycolipids (4, 37) from human kidney. The compositions of fatty acids and long chain bases (15) of fatty acids of human kidney gangliosides are of variable chain length, which is typical of extraneural glycosphingolipids (4, 36). The compositions of fatty acids and long chain bases (15) of gangliosides and neutral glycolipids (4, 37) from human kidney are rather similar, which suggests that these two groups of glycolipids are metabolically related.

The tetrasaccharide core of the two main extraneural-containing gangliosides (II and III) was found to be identical with that found in type II blood group active glycosphingolipids (7) (Fig. 2). The type II glycosphingolipids have a 1-4 linkage between the terminal hexose and the hexosamine. The existence of this linkage in gangliosides II and III was confirmed in the present work by analysis of the partially methylated hexosamine derivatives (Fig. 3). A glycolipid with the tetrasaccharide core of the type II chain attached to ceramide (paragloboside) has been isolated from human erythrocytes (31) and was recently isolated as a specific glycolipid from transformed hamster fibroblasts by Gahmberg and Hakomori (38). Paragloboside probably acts as a precursor in the synthesis of the type II A, B, H, X, and Y antigens (7, 31) and of P, antigen (39, 40). It is also possible that paragloboside may act as a precursor of gangliosides II and III (Fig. 2). The sialic acid moiety of ganglioside II could be added before or after the fucose residue. It is generally assumed that in the synthesis of gangliosides the terminal sialic acid is added last (41, 42) and therefore suggested that the synthesis of X antigen could proceed as the synthesis of ganglioside III.

The content of gangliosides II and III in kidney is rather small (about 1 mg/kg wet weight of each ganglioside). Kidney contains blood group active substances, whose content in kidney is not exactly known. It is, however, in the same range as in erythrocytes (6) and therefore is very small (43-44). The transfer of neuraminic acid to glycolipids with the type II chain has been interpreted as a mode for suppressing the synthesis of blood group active glycosphingolipids in erythrocytes (33) (Fig. 2). The content of gangliosides II and III is sufficient to suggest that a similar mechanism may operate in kidney.

Acknowledgments—The skillful technical assistance of Mrs. Lisa Kuivalainen and Mrs. Hilkka Rönkön is greatly appreciated.

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EXPERIMENTAL PROCEDURE

Antigen determination. Gangliosides were isolated by treatment with trypsin at 37°C for 30 min, except in the case of gangliosides GM1 and GM2. The trypsin was inactivated by heating at 80°C for 5 min before addition of the gangliosides. The trypsin was present at a ratio of 1:100, except for GM1 and GM2, where it was present at a ratio of 1:10. After heating, the gangliosides were allowed to stand at room temperature for 20 min and then centrifuged at 200 g for 10 min. The supernatant was removed and the precipitate was dissolved in 0.05 M Tris-HCl buffer, pH 7.5, containing 1.5% (w/v) SDS and 1.5% (w/v) 2-mercaptoethanol. The gangliosides were isolated by centrifugation at 100,000 g for 1 h, and the supernatant was used for assay.

Table 1. Antigen Titers of Gangliosides

<table>
<thead>
<tr>
<th>Ganglioside</th>
<th>Antigen Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM1</td>
<td>10</td>
</tr>
<tr>
<td>GM2</td>
<td>5</td>
</tr>
<tr>
<td>GM3</td>
<td>1</td>
</tr>
<tr>
<td>GM4</td>
<td>0.5</td>
</tr>
<tr>
<td>GM5</td>
<td>0.1</td>
</tr>
<tr>
<td>GM6</td>
<td>0</td>
</tr>
</tbody>
</table>

Gangliosides were dissolved in 1% (w/v) SDS and 1% (w/v) 2-mercaptoethanol, and the antigen titer was determined by the method of Yagita et al. [1].

Antigenic analysis. Gangliosides were isolated by treatment with trypsin at 37°C for 30 min, except in the case of gangliosides GM1 and GM2. The trypsin was inactivated by heating at 80°C for 5 min before addition of the gangliosides. The trypsin was present at a ratio of 1:100, except for GM1 and GM2, where it was present at a ratio of 1:10. After heating, the gangliosides were allowed to stand at room temperature for 20 min and then centrifuged at 200 g for 10 min. The supernatant was removed and the precipitate was dissolved in 0.05 M Tris-HCl buffer, pH 7.5, containing 1.5% (w/v) SDS and 1.5% (w/v) 2-mercaptoethanol. The gangliosides were isolated by centrifugation at 100,000 g for 1 h, and the supernatant was used for assay.

Table 2. Antigenic Compositions of Gangliosides

<table>
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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>GM1</td>
<td>1</td>
</tr>
<tr>
<td>GM2</td>
<td>0.5</td>
</tr>
<tr>
<td>GM3</td>
<td>0.1</td>
</tr>
<tr>
<td>GM4</td>
<td>0.05</td>
</tr>
<tr>
<td>GM5</td>
<td>0.01</td>
</tr>
<tr>
<td>GM6</td>
<td>0</td>
</tr>
</tbody>
</table>

Gangliosides were dissolved in 1% (w/v) SDS and 1% (w/v) 2-mercaptoethanol, and the antigenic composition was determined by the method of Yagita et al. [1].

RESULTS

Gangliosides 1. The results are summarized in Table 1. The antigen titers of the gangliosides were determined by the method of Yagita et al. [1]. The gangliosides were isolated by treatment with trypsin at 37°C for 30 min, except in the case of gangliosides GM1 and GM2. The trypsin was inactivated by heating at 80°C for 5 min before addition of the gangliosides. The trypsin was present at a ratio of 1:100, except for GM1 and GM2, where it was present at a ratio of 1:10. After heating, the gangliosides were allowed to stand at room temperature for 20 min and then centrifuged at 200 g for 10 min. The supernatant was removed and the precipitate was dissolved in 0.05 M Tris-HCl buffer, pH 7.5, containing 1.5% (w/v) SDS and 1.5% (w/v) 2-mercaptoethanol. The gangliosides were isolated by centrifugation at 100,000 g for 1 h, and the supernatant was used for assay.

Table 3. Antigenic Compositions of Gangliosides

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</tr>
</thead>
<tbody>
<tr>
<td>GM1</td>
<td>1</td>
</tr>
<tr>
<td>GM2</td>
<td>0.5</td>
</tr>
<tr>
<td>GM3</td>
<td>0.1</td>
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<tr>
<td>GM4</td>
<td>0.05</td>
</tr>
<tr>
<td>GM5</td>
<td>0.01</td>
</tr>
<tr>
<td>GM6</td>
<td>0</td>
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