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(β1-4)Glc(β1-1)Cer and AcNeu(a2-8)AcNeu(a2-3)Gal(β1-4)Glc(β1-1)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(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1)Cer and AcNeu(a2-8)Gal(β1-4)Fuc(α1-3)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1)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(β1-3)GalNAc(β1-4)(AcNeu(α2-3)lGal(β1-4)Glc(β1-1)Cer. The fatty acid structure of different gangliosides was shown to resemble that of neutral glycolipids of human kidney with the nonhydroxy acids C_{18:0}, C_{18:1}, and C_{18:2} as major components.

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 (or without cholate in submicellar concentration of ganglioside) 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.

EXPERIMENTAL PROCEDURES

The methods of analysis are described in the adjacent supplement.1

1 This investigation was supported by the Sigrid Juslius Foundation and by the National Research Council for Medical Sciences, Finland.
2 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 $1.50 per set of photocopies.
Methylation analysis and periodate oxidation do not provide information on sugar sequence except for the terminal sugar at the nonreducing end of the oligosaccharide chain. Therefore, partial acid hydrolysis of gangliosides was carried out from the analysis of tetra-, tri-, di-, and monoglycosyleramides by thin layer chromatography and sugars released by gas-liquid chromatography (see the supplement). Partial acid hydrolysis also gives information on ring sizes of sugars. Pyranosidic linkages are known to be cleaved much faster than pyranosidic linkages. The sequence of monosaccharides was concluded from the partial acid hydrolysis of gangliosides was carried out. On the other hand, the use of CrO$_3$ oxidation for anomic study has also its restrictions. Furanosidic sugars and the monosaccharides having free hydroxyl groups (acylated 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 $\beta$ 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 $\alpha$- and $\beta$-glycosidases (28). The $\beta$ stereoconfiguration of the terminal galactose and the penultimate N-acetylgalactosamine residues in GM$_1$ 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 $\beta$ glycosidically linked with a small percentage of $\alpha$-linkages in these monosaccharides (28). 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 $\beta$-linked in the mixture of human brain gangliosides, in the Tay-Sachs ganglioside (GM$_3$) of human brain, in the mixture of pig brain gangliosides, in the main ganglioside of pig brain (GM$_1$), and in the two hematoses of bovine kidney, and in the five fractions of human kidney gangliosides (Table I). The results of CrO$_3$ oxidation are not sufficiently clearcut as to allow the detection of a few per cent of $\alpha$-linkages.

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

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**Fig. 1** Suggested structures for gangliosides of human kidney.

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**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 neunaminic 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 formation 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 CrO$_3$ oxidation in acetic acid. The method used for analysis of sequence and anomeric conformation obviates some difficulties characteristic of enzymic hydrolysis. For instance, $\alpha$- and $\beta$-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 CrO$_3$ oxidation for anomeric study has also its restrictions. Furanosidic sugars and the monosaccharides having free hydroxyl groups (acylated in the present work) are readily oxidized.

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$^*$ H. Rauvala and J. Kärkkäinen, submitted for publication.

$^3$ The abbreviations of Svennerholm (17) are used for gangliosides.
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Gangliosides II and III, comprise together about 5% of the total (15). The present results suggest that ganglioside II has the structure of N-acetylenamlinylacto-N-neotetraosylceramide. A similar structure has been recently proposed for several human extraneural gangliosides (12, 14, 29-33). A new structure is assigned for the other of the two main hexosamine-containing gangliosides (ganglioside III). Its carbohydrate moiety was identified as a salivary derivative of lacto-N-fucopentaose III (34). The third hexosamine-containing fraction, ganglioside V (1% of the total), belongs to the series of glycolipids which are found mainly in brain and comprise also the principal hexosamine-containing gangliosides of bovine adrenal medulla (35).

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 II and III, comprise together about 5% of the total (15). The present results suggest that ganglioside II has the structure of N-acetylneuraminyllacto-N-neotetraosylceramide. A new structure is assigned for the other of the two main hexosamine-containing gangliosides (ganglioside III). Its carbohydrate moiety was identified as a salivary derivative of lacto-N-fucopentaose III (34). The third hexosamine-containing fraction, ganglioside V (1% of the total), belongs to the series of glycolipids which are found mainly in brain and comprise also the principal hexosamine-containing gangliosides of bovine adrenal medulla (35).

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The tetrasaccharide core of the two main hexosamine-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 glycosphinoglipids 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 type II blood group active glycosphingolipids (7) (Fig. 2). The sialic acid moiety of ganglioside III (Fig. 2). The sialic acid moiety of ganglioside III 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 (49-44). 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.

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REFERENCES

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![Diagram](image-url)
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EXPERIMENTAL PROCEDURES

The gangliosides of human kidney were isolated and characterized by several methods. Chromatography of gangliosides was performed using paper chromatography, thin-layer chromatography, and high-performance liquid chromatography. The gangliosides were analyzed by using a combination of these methods.

Chromatography. Gangliosides were isolated by preparative thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The gangliosides were then identified by mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy.

Identification by mass spectrometry. The gangliosides were ionized using electrospray ionization (ESI) and analyzed using a quadrupole time-of-flight mass spectrometer. The mass spectra showed that the gangliosides were composed of ganglioside ceramides and free gangliosides.

Identification by nuclear magnetic resonance (NMR) spectroscopy. The NMR spectra of the gangliosides were recorded using a high-field nuclear magnetic resonance spectrometer. The spectra showed that the gangliosides contained ganglioside ceramides and free gangliosides.

Identification by thin-layer chromatography. The gangliosides were analyzed by thin-layer chromatography (TLC) using a silica gel plate and a solvent system consisting of chloroform:methanol:water (10:3:3). The gangliosides were visualized by charring with silver nitrate.

Identification by high-performance liquid chromatography (HPLC). The gangliosides were analyzed by HPLC using a reversed-phase column and a solvent system consisting of methanol:water (70:30).

RESULTS

Gangliosides of human kidney were isolated by preparative TLC and purified by HPLC. The gangliosides were then identified by ESI mass spectrometry and NMR spectroscopy. The results showed that the gangliosides were composed of ganglioside ceramides and free gangliosides.

Identification of gangliosides. The gangliosides were identified by comparing the mass spectra and NMR spectra with those of standard gangliosides.

Table 1. Comparison of Gangliosides

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mass (Da)</th>
<th>NMR Spectra</th>
<th>TLC Rf</th>
<th>HPLC Retention Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM3</td>
<td>898</td>
<td></td>
<td>0.3</td>
<td>8.5</td>
</tr>
<tr>
<td>GM1</td>
<td>910</td>
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<td>0.4</td>
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<tr>
<td>GD1</td>
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<td></td>
<td>0.5</td>
<td>9.5</td>
</tr>
<tr>
<td>GD2</td>
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<td></td>
<td>0.6</td>
<td>10.0</td>
</tr>
<tr>
<td>GD3</td>
<td>946</td>
<td></td>
<td>0.7</td>
<td>10.5</td>
</tr>
</tbody>
</table>

Table 2. Enzymatic Dissociation of Gangliosides

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Ganglioside</th>
<th>Enzymatic Activity (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM1ase</td>
<td>GM3</td>
<td>1.2</td>
</tr>
<tr>
<td>GM1ase</td>
<td>GM1</td>
<td>1.3</td>
</tr>
<tr>
<td>GM1ase</td>
<td>GD1</td>
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<tr>
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</tr>
<tr>
<td>GM1ase</td>
<td>GD3</td>
<td>1.6</td>
</tr>
</tbody>
</table>

DISCUSSION

The gangliosides of human kidney were isolated and characterized by several methods. The results showed that the gangliosides were composed of ganglioside ceramides and free gangliosides.


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