THE FUCOGANGLIOSIDE OF HUMAN KIDNEY

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1. Introduction

Gangliosides of human kidney have been isolated and partially characterized [1]. One of these gangliosides contained fucose, galactose, glucose, *N*-acetylglucosamine and *N*-acetylneuraminic acid in the molar ratio of 1:2:1:1:1 respectively. It was homogeneous on thin-layer chromatography and it was therefore proposed that this ganglioside represents a new type of glycolipid structure with neuraminic acid and fucose residues in the same carbohydrate chain [1]. In the present work the carbohydrate moiety of this ganglioside was studied by methylation, partial acid hydrolysis and neuraminidase treatment. Evidence is presented that this ganglioside is a sialized derivative of the so-called X antigen isolated by Yang and Hakomori [2].

2. Experimental

2.1. *Materials*

Gangliosides of human kidney were isolated and fractionated as described before [1]. Hematosides of bovine kidney [3] were made available by Dr Puro (Helsinki, Finland). Lactosylceramide was prepared from hematoside by neuraminidase treatment or by weak acid hydrolysis (0.1 M HCl, 100°C, 1 h). Glucosylceramide was obtained from hematoside [3] by weak acid hydrolysis (0.1 M HCl, 100°C, 3 h). Fucose, galactose, glucose, *N*-acetylgalactosamine, *N*-acetylglucosamine, inositol, *N*-acetylneuraminic acid and lactose were purchased from commercial sources. The following sugars were gifts from Dr Gauhe (Heidelberg, Germany): βGal-(1→4)-GlcNAc*, βGal-(1→3)-GlcNAc, αGal-(1→6)-GlcNAc, αFuc-(1→2)-βGal-(1→4)-Glc (2′-fucosyllactose), βGal-(1→3)-βGlcNAc-(1→3)-βGal-(1→4)-Glc (lacto-N-tetraose), αFuc-(1→2)-βGal-(1→3)-βGlcNAc-(1→3)-βGal-(1→4)-Glc (lacto-N-fucopentose I) and βGal-(1→3)-[αFuc-(1→4)]-βGlcNAc-(1→3)-βGal-(1→4)-Glc (lacto-*N*-fucopentaose II).

2.2. *Analytical methods*

Tlc was carried out on 0.25 mm silica gel G plates. The solvent was chloroform/methanol/2.5 M NH_4OH (60/35/8, v/v/v). Glc was performed with a Perkin-Elmer 900 gas chromatograph. The columns were 2.2% SE-30 and 1% OV-225 (2 m × 3 mm, i.d.). Glc–ms was performed with a Varian 1700 gas chromatograph coupled to a Varian MAT CH-7 mass spectrometer equipped with Spectro System 100 ms data processing system. Ionization potential was 70 eV, and ionization current was 300 μA. The mass range studied was from m/e 40 to m/e 800. The columns were 1% SE-30 and 1% OV-225.

2.3. *Permethylation and identification of partially methylated sugars*

40–100 μg of ganglioside or neutral glycolipid obtained from ganglioside by neuraminidase treatment was methylated with methyl iodide in dimethylsulfoxide in the presence of dimethylsulfinyl carbanion [4]. The permethylated product was degraded, reduced and acetylated as described before for analysis of sugar composition in glycolipids [2]. The resulting partially methylated alditol acetates were analyzed by glc on 1% OV-225 column [5] and by glc–ms on 1%

* *Abbreviations.* Fuc, fucose; Gal, galactose; Glc, glucose; GlcNac, *N*-acetylglucosamine; NeuNAc, *N*-acetylneuraminic acid; Cer, ceramide; glc, gas-liquid chromatography; ms, mass-spectrometry; tlc, thin-layer chromatography.

OV-225 or 1% SE-30 column (1% SE-30 at 190°C was preferred in glc–ms for analysis of amino sugar derivatives because of lower column bleed). Identification of methylation products was based on comparison with standards (obtained from methylation of reference sugars) and on fragmentation data [6,7].

2.4. *Partial acid hydrolysis*

30–50 μg of ganglioside was hydrolyzed in 0.1 ml of 0.1 M HCl at 100°C for 1 h and 3 h. Glycolipid was partitioned into the organic phase by addition of 4 vols of chloroform/methanol 2/1 (v/v) and analyzed on tlc. The liberated monosaccharides were analyzed from the upper phase after trimethylsilylation [8] on 2.2% SE-30 by a program from 180°C to 260°C (the program rate was 4°C/min). Disaccharides were analyzed isothermally at 260°C or 280°C. Peak identification was based on reference mono- and disaccharides.

2.5. *Neuraminidase treatment*

80 μg of ganglioside was dissolved in 0.5 ml of 0.01 M Tris-acetate buffer [9], pH 6.8, and 25 μl *Vibrio cholerae* neuraminidase (Calbiochem, San Diego, USA) was added. Incubation was performed at 37°C for about 20 h. Glycolipid was partitioned into the lower Folch phase [10,11] by 0.1 M $CaCl_2$ at pH 2.2. Half of the lower phase was evaporated to dryness with nitrogen, and methylated. Glycolipid and neuraminic acid were also analyzed on tlc.

3. Results

3.1. *Homogeneity of glycolipid*

The glycolipid fraction containing fucose, galactose, glucose, *N*-acetylglucosamine and *N*-acetylneuraminic acid has been shown to be homogeneous on tlc in several different solvents [1]. However, tlc is not a safe criterion of homogeneity for glycolipids having more than five monosaccharide units in their carbohydrate chains [12]. In this case the possibility that fucose could originate from some impurity, e.g. neutral glycolipid or glycopeptide, must be especially considered. It was shown that this ganglioside fraction does not contain free fucose. This is evident from the fact that not a trace of fucose was found in glc, when an aliquot of ganglioside fraction was directly trimethylsilylated and analyzed by glc. In Folch partition, when distilled water was used, fucose was found in the upper phase by mild acid hydrolysis, trimethylsilylation and glc. When 0.1 M $CaCl_2$ (buffered to pH 2.2 with glycine-HCl) was used instead of distilled water in Folch partition, the bulk of fucose was found in the lower phase. This kind of behaviour in Folch partition is typical of gangliosides, and it can be concluded that fucose is lipid bound, and cannot originate from a neutral fucolipid (which would be mainly partitioned into the lower phase also when distilled water is used). Further, the neutral glycolipid obtained from ganglioside by neuraminidase treatment was also homogeneous on TLC (and moved somewhat slower than the neutral glycolipid, which was obtained from ganglioside by weak acid hydrolysis, which detached fucose, in addition to neuraminic acid).

3.2. *Carbohydrate linkages*

Carbohydrate linkages were studied by methylation. Methylation products of the intact ganglioside were identified as acetates of 2, 3, 4-tri-*O*-methylfucitol, 2, 4, 6-tri-*O*-methylgalactitol, 2, 3, 6-tri-*O*-methylglucitol and 6-mono-*O*-methyl-*N*--methylacetamidoglucitol (fig.1). 2, 4, 6-tri-*O*-methylgalactitol acetate and 2, 3, 6-tri-*O*-methylglucitol acetate were found in the molar ratio of 2:1 respectively. Methylation after neuraminidase treatment gave 2, 3, 4, 6-tetra-*O*-methylgalactitol acetate, 2, 4, 6-tri--*O*-methylgalactitol acetate and 2, 3, 6-tri-*O*-methylglucitol acetate in the molar ratio of 1:1:1. This experiment indicates that neuraminic acid is attached to C3 of the terminal galactose molecule. The methylated hexosamine of the intact ganglioside was shown by glc-ms to be 6-mono-*O*-methylated glucosamine derivative. After weak acid hydrolysis, βGal-(1→4)-GlcNAc was identified by glc. In the conditions used βGal--(1→4)-GlcNAc, βGal-(1→3)-GlcNAc and αGal-(1→6)-GlcNAc were clearly different in glc. It can therefore be concluded that fucose is attached to C3 of *N*-acetylglucosamine.

3.3. *Sequence of monosaccharide units*

In the intact ganglioside neuraminic acid and fucose are terminal. This is indicated by the fact that neuraminic acid was susceptible to the action of an end group hydrolase, neuraminidase, and the fact that fucose gave a fully methylated fucopyranose derivative. Neuraminidase treatment disclosed a terminal

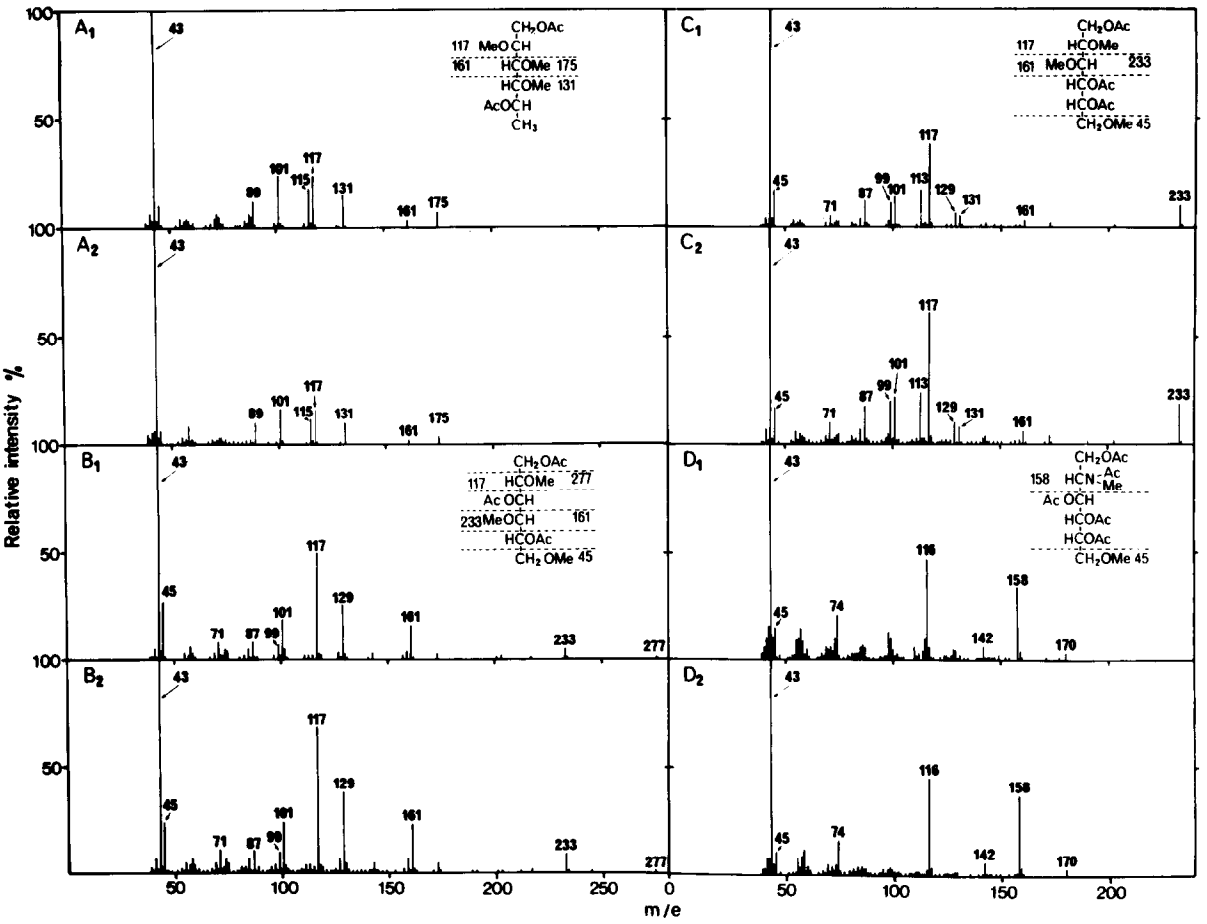

Fig.1. Methylation products of the fucose-containing ganglioside of human kidney. A_1-D_1, methylation products from standard sugars; A_2-D_2, methylation products from ganglioside. A_1, A_2, 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylfucitol; B_1, B_2, 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylgalactitol; C_1, C_2, 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol; D_1, D_2, 1,3,4,5-tetra-*O*-acetyl-6-mono-*O*-methyl-2-*N*-deoxymethylacetamidoglucitol.

galactose, which was shown by methylation (the fully methylated galactopyranose derivative was obtained). After weak acid hydrolysis (1 or 3 h), βGal-(1–4-Glc-Cer (lactosylceramide) was identified on tlc. A trace of glucosylceramide was also identified on tlc (weak acid hydrolysis for 3 h). A disaccharide, identical with βGal-(1–4)-GlcNAc in glc, was obtained in weak acid hydrolysis (1 or 3 h). Therefore, the tetraglycosyl-ceramide core of the ganglioside is Gal-GlcNAc-Gal-Glc-Cer. Neuraminic acid is attached to the terminal galactose and fucose to *N*-acetylglucosamine (see above).

Combining the results of permethylation, weak acid hydrolyses and neuraminidase treatment, the structure of the 'fucoganglioside' of human kidney is:

Gal-(1–4)-GlcNAc-(1–3)-Gal-(1–4)-Glc-(1–1)-Cer
3 3
↑ ↑
2 1
NeuNAc Fuc

4. Discussion

The isolated glycolipid has a new carbohydrate structure. As to my knowledge, a glycolipid with neuraminic acid and fucose residues in the same carbo-

hydrate chain has not been isolated before. An H active oligosaccharide containing fucose, galactose, glucose, galactosamine and neuraminic acid has been found from gangliosides of bovine liver [13].

A neutral glycosphingolipid with similar structure, the so-called X antigen, has been isolated from human adenocarcinoma by Yang and Hakomori [2,14] and recently also from hog gastric mucosa by Slomiany et al. [15]. The present ganglioside can probably be regarded as a sialized derivative of this so-called X antigen. It was proposed by Yang and Hakomori [2] that the X antigen occurs in tumour tissue in higher quantities than in normal tissue. They did not detect any activity of X antigen in water-soluble fractions of human parenchymatous organs, e.g. kidney. It is possible that the X antigen occurs in a neuraminic acid-containing form in normal tissue, and cannot therefore be detected by immunological techniques, because neuraminic acid is known to mask antigenicity of cell surface compounds [16].

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