Structural characterization of a blood group A heptaglycosylceramide with globo-series structure

The major glycolipid based blood group A antigen of human kidney

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Received 19 October 1984

A blood group A glycosphingolipid with the globo-series structure has been isolated from human kidney and structurally characterized. The structure was shown by mass spectrometry and proton NMR spectroscopy of the intact permethylated and permethylated-reduced derivatives together with degradation studies to be, GalNAcα1→3Gal(2←1αFuc)β1→3GalNAcβ1→3Galα1→4Galβ1→4Glcβ1→1Ceramide. This glycolipid reacts with both polyclonal and monoclonal anti-A blood group typing antisera and it is the major glycolipid based blood group A antigen present in the human kidney.

Blood group A *Glycolipid* *Globo-series* *Human kidney*

1. INTRODUCTION

A large number of blood group A glycosphingolipids have been isolated and structurally characterized from human and animal tissues (review [1,2]). Blood group A glycolipids present in human tissues are based upon type 1 (Galβ1→3GlcNAc) and/or type 2 (Galβ1→4GlcNAc) carbohydrate chains [1,2] while other types of core saccharides carrying the A trisaccharide have been identified in animal tissues [1–3]. This paper describes the structural characterization of a novel blood group A heptaglycosylceramide isolated from human blood group A kidneys. This glycolipid has the A trisaccharide linked β1→3 to the galactosamine of globotetraosylceramide and represents a new type of core saccharide based A antigen in human tissue. When this work was completed, we learned that the same compound had been isolated from human A_1 erythrocytes (S. Hakomori, personal communication).

The data were presented at the XIIth International Carbohydrate Symposium, Utrecht, The Netherlands, July 1984

2. EXPERIMENTAL

Kidneys were obtained from blood group A human individuals at autopsy. The total non-acid glycolipid fraction was prepared as in [3–6]. The major part of the glycolipids with 1–4 sugar residues was removed by repeated silicic acid chromatography [3] and the polar glycolipid fraction obtained (fig.1, lane B) was further separated on an Iatrobeads column (Iatron, Tokyo) eluted by a continuous gradient of chloroform-methanol-water (from 65:25:4 to 50:40:10 followed by a final elution with 40:40:12, by vol.). After separation by thin-layer chromatography, the fractions were combined into 8 partly purified fractions for structural analysis. The fraction characterized here (fig.1, lane C) had a thin-layer mobility as a heptaglycosylceramide.

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00145793/85/$3.30

The permethylated and permethylated-reduced ($LiAlH_4$) intact glycolipid fractions were analysed by mass spectrometry [7,8] on a ZAB 2F high resolution instrument (VG Instruments) and by 270 MHz proton NMR spectroscopy [9–11].

The carbohydrate composition and sugar linkage positions were established by degradation of the native, permethylated and permethylated-reduced derivatives as in [6,12,13]. The degradation products were identified by gas chromatography and gas chromatography-mass spectrometry. Blood group A activity of the native glycolipid fraction was tested by hemagglutination-inhibition [14] using a commercial human polyclonal anti-A antiserum (Biol. Corp., West Chester). The reactivity against a mouse monoclonal anti-A antibody (Biotest, Frankfurt) was tested by immunostaining on the thin-layer plate [15,16].

3. RESULTS

The weight of the total non-acid glycolipid fraction (fig.1, lane A) isolated from the human blood group A kidneys was 2.9 g corresponding to 5.6 mg per g dry tissue weight. Tri- and tetraglycosylceramides were the major glycolipids present together with mono- and diglycosylceramides as reported in [17]. In addition, two bands were seen migrating as glycolipids having 5 and 7 sugar residues, respectively. The pentaglycosylceramide fraction consisted of 75% of globotetraosylceramide with an additional terminal Galβ1→3 residue and 25% of the X-5 glycolipid (type 2 carbohydrate chain isomer of the Lewis[a] pentaglycosylceramide) ([18]; to be published). The weight of the heptaglycosylceramide fraction (fig.1, lane C) was 5.5 mg and it contained one major component together with small amounts of tetra- and pentaglycosylceramides. As acetylated derivatives, glycolipids sometimes change their relative chromatographic migration [3,6,12]. Acetylation of the heptaglycosylceramide fraction revealed one major band migrating just behind globotetraosylceramide and with an identical R_f value as a type 1 chain blood group A hexaglycosylceramide.

Mass spectrometry of the permethylated-reduced heptaglycosylceramide fraction (fig.2) shows a series of intense peaks at *m/z* 1764–1876 due to ions containing the whole saccharide chain

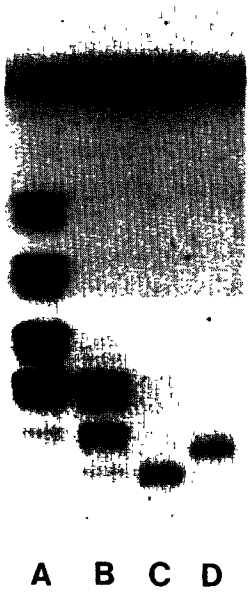

Fig.1. Thin-layer chromatogram of total non-acid glycolipids isolated from kidneys of blood group A human individuals (lane A). Lane B shows a polar glycolipid fraction obtained from the total fraction. This fraction was further separated by gradient elution and the fractions containing the heptaglycosylceramide were combined (lane C). Reference A_6-1 glycolipid GalNAcα1→3Gal(2←1αFuc)β1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Ceramide (lane D) was from rat small intestine [3]. The thin-layer plate was HPTLC, Kiselgel 60 (Merck), solvent was chloroform–methanol–water, 60:35:8 (by vol.), and detection was anisaldehyde [4].

and 16:0–24:0 non-hydroxy fatty acids. The corresponding fragment for the 24:0 hydroxy fatty acid species is seen at *m/z* 1906. The ion at *m/z* 1838 originates from the hydroxy fatty acid species as shown in the formula. Molecular ions for the trihydroxy long chain base (t18:0, phytosphingosine) in combination with 24:0 non-hydroxy and hydroxy fatty acids are present at *m/z* 2160 and 2190, respectively. Primary carbohydrate sequence ions are found at *m/z* 157, 189, 246, 262, 624, 855, 871, 1059 and 1467. Secondary sequence ions containing the fatty acid and the inner 3 and 4 sugar residues are present at *m/z* 1022 and 1253 for the 24:0 non-hydroxy and at *m/z* 1052 and 1283 for the 24:0 hydroxy fatty acid species, respectively. The presence of type 1 or type 2 carbohydrate

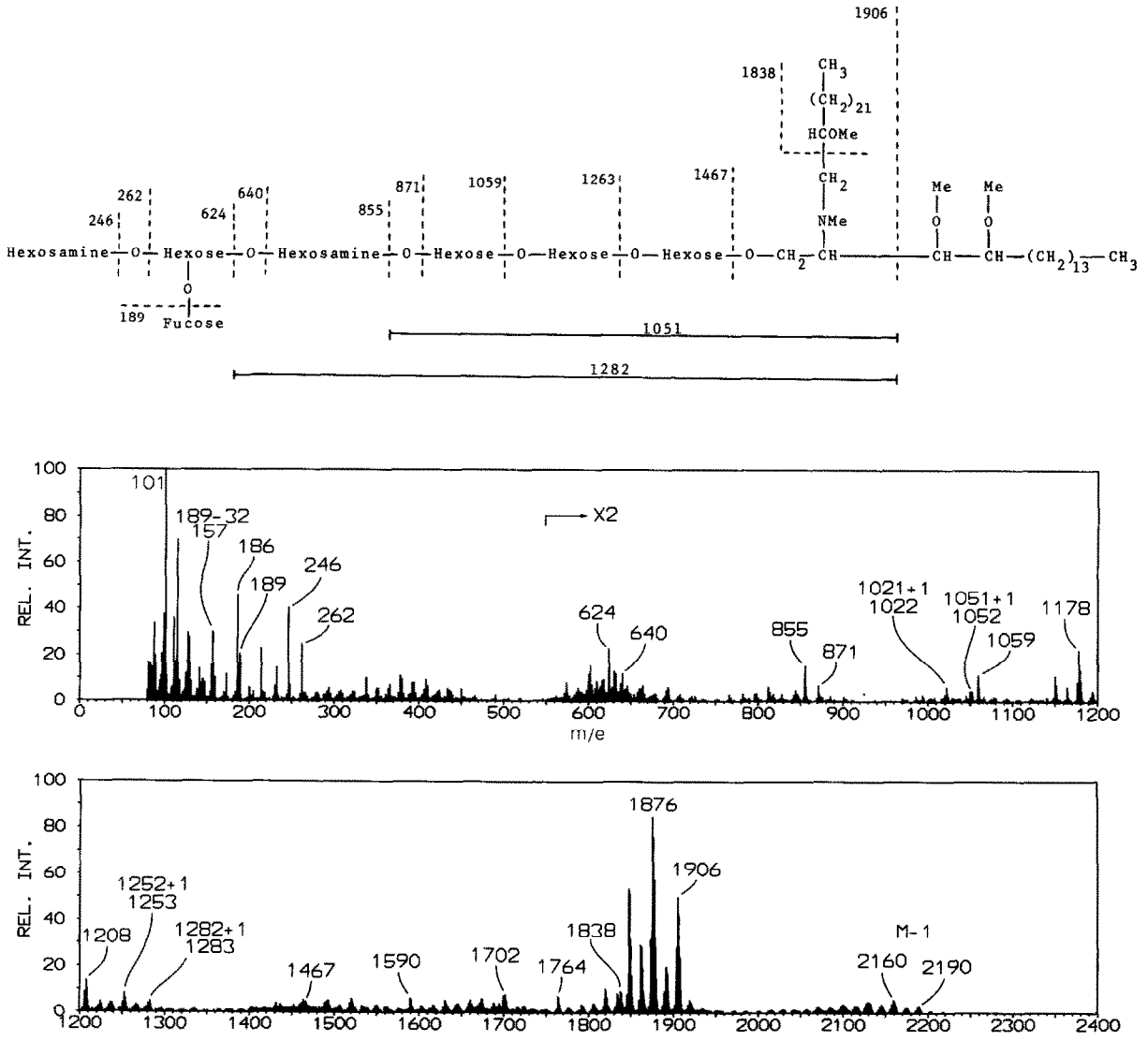

Fig.2. Mass spectrum and simplified formula for interpretation of the permethylated-reduced heptaglycosylceramide fraction isolated from human kidney. The conditions of analysis were: sample amount 15 μg, electron energy 45 eV, trap current 200 μA, acceleration voltage 8 kV and ion source temperature 340°C. Peaks below *m/e* 80 were not reproduced.

chains in glycolipids can be established by mass spectrometry [5,7,12,19]. For reduced derivatives, a substitution at carbon atom 3 of the hexosamine (as for type 1 chains) results in a rearrangement ion containing the fatty acid, the inner hexoses and part of the hexosamine [19]. In this case, corresponding fragments are present at *m/z* 1178 (1876 – 624 – 74) and at *m/z* 1208 (1906 – 624 – 74) indicating a substitution at C3 of the hexosamine.

In the mass spectrum of the permethylated derivative (not shown) primary carbohydrate sequence ions were present at *m/z* 157, 189 (terminal fucose), *m/z* 228, 260 (terminal hexosamine), *m/z* 606, 638 (trisaccharide), *m/z* 851, 883 (tetrasaccharide), *m/z* 1087 (pentasaccharide), *m/z* 1291

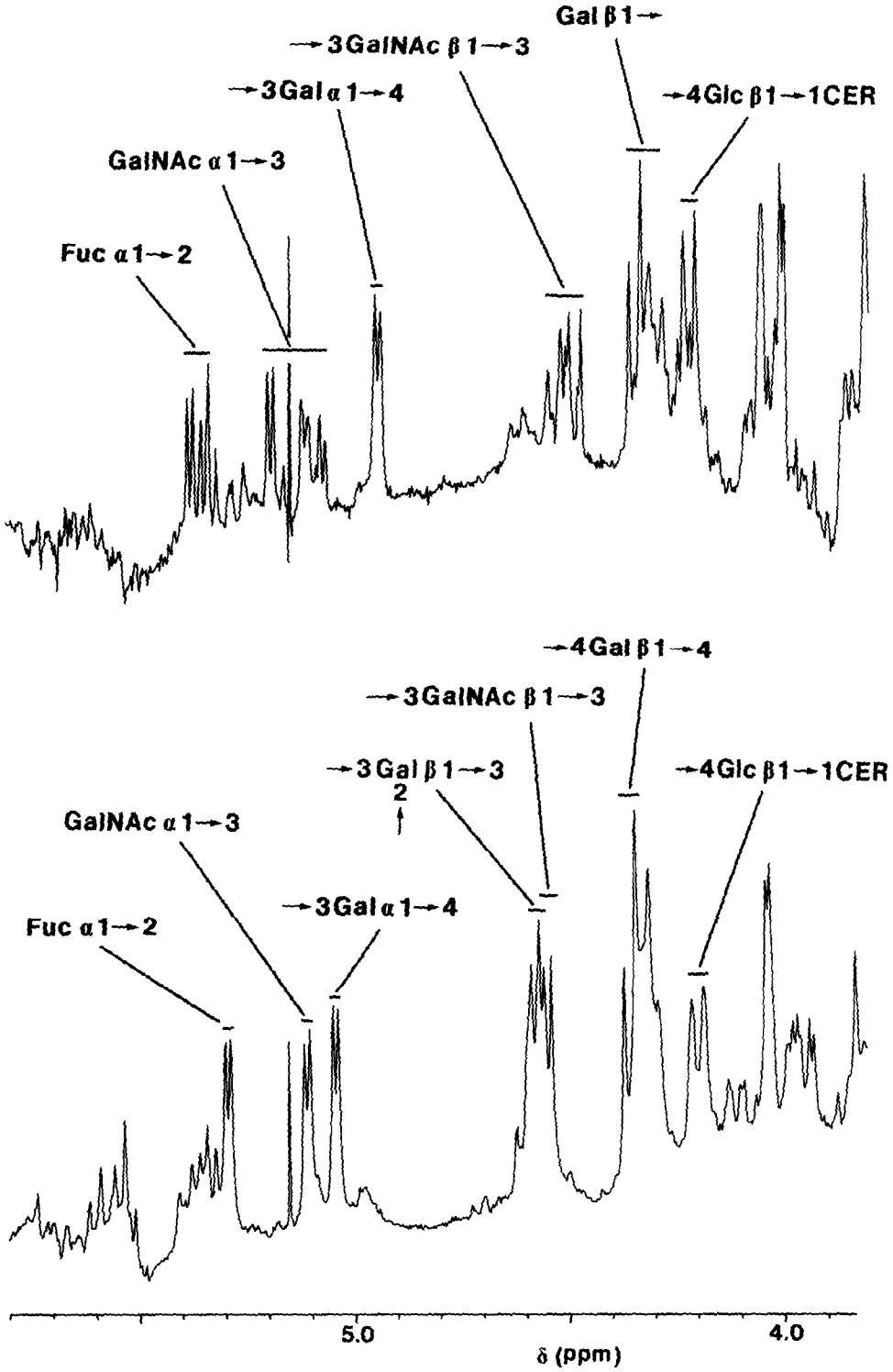

Fig.3. 270-MHz proton NMR spectra of the permethylated (top) and permethylated-reduced (bottom) heptaglycosylceramide fraction isolated from human kidney. The amounts of substance analyzed were: (A) 2.5 mg and (B) 2.0 mg dissolved in 0.45 ml C^2HCl_3. (A) 3400 pulses and (B) 4400 pulses at 40°C.

(hexasaccharide) and *m/z* 1568 (complete saccharide chain plus part of the ceramide). Ceramide ions were present at *m/z* 548–660 for dihydroxy long chain base (d18:1, sphingosine) in combination with 16:0–24:0 non-hydroxy fatty acids and at *m/z* 692 and 722 for t18:0 base in combination with 24:0 non-hydroxy and hydroxy fatty acids, respectively. The specific ions for d18:1 and t18:0 bases at *m/z* 364 and 396 were of equal intensity.

The mass spectra also revealed the presence of small amounts of other glycolipids. The peaks at *m/z* 1590–1702 in fig.2 originate from a blood group A hexaglycosylceramide [3]. In spectra recorded at lower ion source temperature, fragments indicating globotetraosyl-, globopentaosyl- and difucosyl blood group A heptaglycosylceramides were present. In addition, fragments originating from the blood group H hexaglycosylceramide of the globo-series [19] were also found. These fragments were, however, more intense in the spectrum of the hexaglycosylceramide fraction eluted in front of the heptaglycosylceramides on the Iatrobeads column (see section 2). The total amount of the contaminants was 10–15% as calculated from the ion intensities.

In the proton NMR spectrum of the permethylated derivative (fig.3, top), two signals from the H-1 of the terminal α-fucose and α-galactosamine are found at 5.39, 5.35 and 5.2–5.1 ppm, respectively. In the spectrum of the reduced derivative (fig.3, bottom) the signal shave slid together giving sharp signals at 5.30 ppm for the α-fucose and 5.12 ppm for the α-galactosamine with small coupling constants ($J_{1,2}$ = 2.8 and 3.3 Hz, respectively). The same phenomena have been noted for other blood group A and B

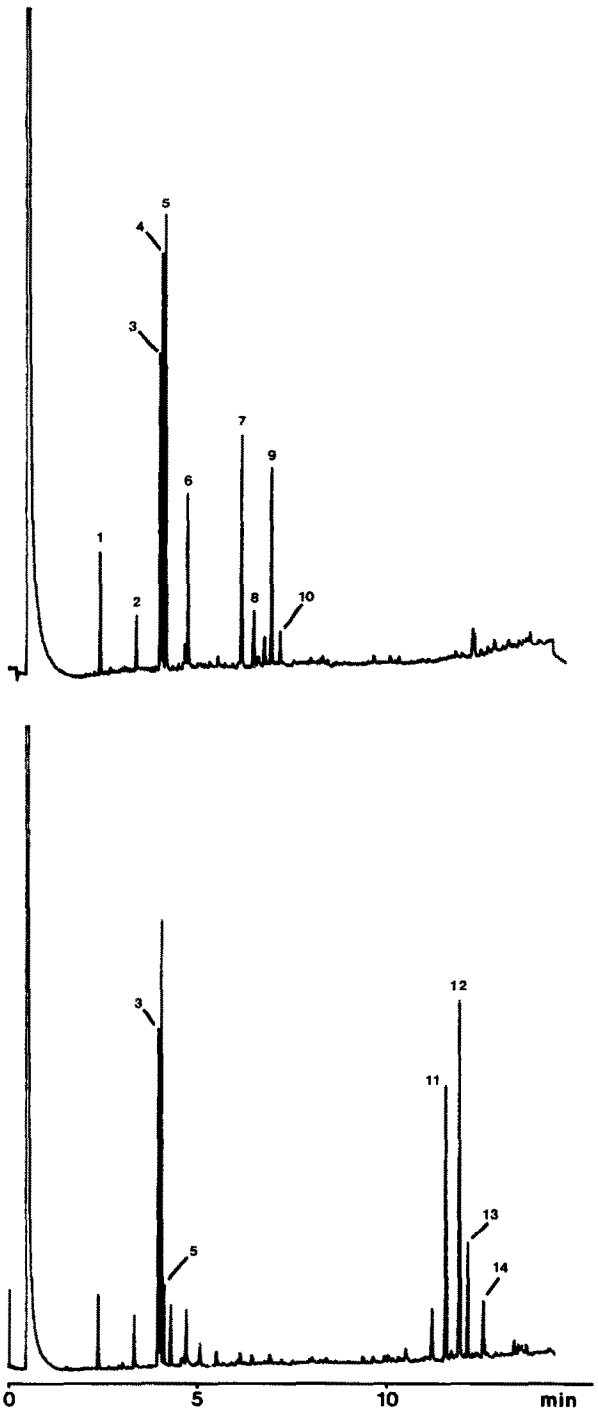

Fig.4. Gas chromatograms of the products obtained by degradation of the permethylated (top) and permethylated-reduced (bottom) heptaglycosylceramide fraction isolated from human kidney. The identity of the peaks was established by mass spectrometry and comparison with reference products to be: (1) Fuc1→, (2) Gal1→, (3) →4Gal1→, (4) →4Glc1→, (5) →3Gal1→, (6) $\rightarrow^{3}_{2}$Gal1→, (7) GalNAc1→, (8) →4GlcNAc1→, (9) →3GalNAc1→, (10) $\rightarrow^{4}_{3}$GlcNAc1→, (11) GalNAc1 →3Gal1→, (12) →3GalNAc1→3Gal1→, (13) →4GlcNAc1→3Gal1→ and (14) $\rightarrow^{4}_{3}$GlcNAc1→ 3Gal1→. An open tubular column coated with cross-linked nonpolar SE-54 as stationary phase was used. The temperature was programmed from 150 to 300°C at a rate of 10°C/min.

glycolipids [5,10,11]. The β-signal of the galactose linked to the internal galactosamine is found in the β-hexose region at 4.30 ppm for the permethylated derivative and has moved downfield to 4.59 ppm ($J_{1,2}$ = 8.1 Hz) in the spectrum of the reduced derivative. A similar downfield shift is found in spectra of glycolipids based on type 1 carbohydrate chains but not for type 2 chain isomers [5,11]. The β-signal for the internal galactosamine is present at 4.50 and 4.56 ppm ($J_{1,2}$ = 8.1 Hz) for the two derivatives, respectively. The sharp signals having a small coupling constant present at 4.95 ppm ($J_{1,2}$ = 3.5 Hz) for the permethylated derivative and at 5.05 ppm ($J_{1,2}$ = 2.8 Hz) for the reduced derivative originate from an internal α-galactose residue and are in accordance with the spectra of globotetraosylceramide [9]. The two β-signals for the remaining galactose and the glucose linked to ceramide are found at 4.30 and 4.21 ppm for the permethylated derivative and at 4.36 and 4.21 ppm for the reduced derivative.

The gas chromatogram of the products obtained by degradation of the native glycolipid fraction revealed 5 peaks of acetylated alditols corresponding to fucose (10%), galactose (43%), glucose (21%), galactosamine (22%), and glucosamine (4%).

The major degradation products obtained from the permethylated glycolipid fraction were identified by gas chromatography (fig.4, top) and mass spectrometry as the acetates of 2,3,4-trimethylfucitol (Fuc1→), 2,3,6-trimethylgalactitol (→4Gal1→), 2,3,6-trimethylglucitol (→4Glc1→), 2,4,6-trimethylgalactitol (→3Gal1→), 4,6-dimethylgalactitol ($\rightarrow^{3}_{2}$Gal1→), 3,4,6-trimethyl-2-*N*-methylacetamido-2-deoxygalactitol (GalNAc1→) and 4,6-dimethyl-2-*N*-methyl-acetamido-2-deoxygalactitol (→3GalNAc1). In addition, minor peaks of 2,3,4,6-tetramethylgalactitol (Gal→), 3,6-dimethyl-2-*N*-methylacetamido-2-deoxyglucitol (→4GlcNAc1→) and 6-methyl-2-*N*-methylacetamido-2-deoxyglucitol ($\rightarrow^{4}_{3}$GlcNAc1→) were present. These peaks originate from the minor glycolipid components found in the mass spectra. For the permethylated-reduced derivative (fig.4, bottom), the amino sugars together with →3Gal1→ and $\rightarrow^{3}_{2}$Gal1→ have disappeared and instead peaks with retention times corresponding to disaccharides are present. This is caused by the reduced amide group in position 2 of the amino sugars which stabilizes the adjacent glycosidic bond to the next sugar giving rise to disaccharides or trisaccharides [5,12,13]. The two major peaks were identified as GalNAc1 →3Gal1→ and →3GalNAc1 →3Gal1→ and the minor ones as →4GlcNAc1 →3Gal1→ and $\rightarrow^{4}_{3}$GlcNAc1→3Gal1→.

In conclusion, the structural studies of the heptaglycosylceramide fraction have shown one major glycolipid component with the structure GalNAcα1→3Gal(2←1αFuc)β1→3GalNAcβ1→3 Galα1 →4Galβ1→4Glcβ1→1Ceramide. The ceramide part was made up of both 18:1 dihydroxy- and 18:0 trihydroxy long chain bases in combination with 16:0–24:0 non-hydroxy and 22:0–24:0 hydroxy fatty acids. The total amount of this component in the kidney was estimated to 16 μg per g dry tissue weight.

ceramide part was made up of both 18:1

Table 1

Blood group A activity of the heptaglycosylceramide fraction isolated from human kidney

Antigen	Dilution of anti-A antiserum							
	1:0	1:1	1:2	1:4	1:8	1:16	1:32	1:64
Saline control	4+	4+	4+	4+	4+	3+	1+	–
Kidney glycolipid	3+	–	–	–	–	–	–	–
A_6-1 glycolipid	3+	1+	–	–	–	–	–	–

The test was performed as an inhibition of hemagglutination of human blood group A red cells by anti-A antiserum [14]. The glycolipids were incorporated in liposomes (cholesterol 25 μg, hydrated lecithin 50 μg, glycolipid 50 μg). 4+, complete agglutination; –, no agglutination

This glycolipid fraction had an identical blood group A activity as a pure blood group A hexaglycosylceramide based on a type 1 chain, when tested against a polyclonal anti-A antiserum (table 1). It also reacted with a mouse monoclonal anti-A antiserum when tested by immunostaining on the thin-layer plate (not shown).

4. DISCUSSION

The extension of globotetraosylceramide to blood group H and A structures represents a new type of core saccharide based H and A glycolipid antigen in human tissues in addition to the type 1 and type 2 carbohydrate chains. A similar type of saccharide having the H and A terminals linked $\beta 1 \rightarrow 3$ to galactosamine has been identified in oligosaccharides liberated from human ovarial cyst glycoproteins [20,21]. This type of saccharide was referred to as a type 3 carbohydrate chain [21].

The blood group H hexaglycosylceramide has been found in human meconium [19], and teratocarcinoma cells [22]. It was also present in the kidneys in small amounts as seen by mass spectrometry (see section 3). It has recently been isolated from human O erythrocytes [23]. The A heptaglycosylceramide was isolated from human A_1 erythrocytes but was found to be absent in A_2 erythrocytes (S. Hakomori, personal communication). These new blood group H and A glycolipid structures are interesting examples of an earlier unknown interaction between the blood group P and ABO systems in adult human tissues. The biosynthetic pathway for these components is currently under investigation (to be published).

This novel A glycolipid makes up about half of the glycolipid based blood group A antigens present in human A kidneys in contrast to erythrocytes where it is only a very minor compound compared with other A glycolipids (S. Hakomori, personal communication). The cellular localization of this A antigen in the kidney and its role as transplantation antigen in blood group incompatible kidney transplantation (A_2 kidneys to O recipients [24]) is interesting to know. This may now be elucidated by use of monoclonal antibodies against these structures and patient sera in combination with immunohistology and immunostaining of glycolipids on the thin-layer plate [15,16].

ACKNOWLEDGEMENTS

We are indebted to I. Pascher for help with the derivatizations. The work was supported by grants from the Swedish Medical Research Council (nos 7131 and 3967) and the Swedish Natural Science Research Council (no.K4611-103).

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