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Blood group type glycosphingolipids of human kidneys. Structural characterization of extended globo-series compounds

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Blood group type glycosphingolipids present in kidneys of blood group A and B human individuals have been isolated and structurally characterized by mass spectrometry, proton NMR spectroscopy, degradation studies and by their reactivity with various monoclonal antibodies and *Escherichia coli* bacteria. The two major complex glycolipids present in the blood group A and B kidneys were globopentaosylceramide (IV^{3}Galβ-Gb$_4$Cer) and the X pentaglycosylceramide (III^{3}Fucα-nLc$_4$Cer). The major blood group A glycolipid in the blood group A kidneys was based on the type 4 chain (globo-series). There were also small amounts of the type 2 chain and trace amounts of the type 1 and type 3 chain based A glycolipids. In addition, the blood group H type 4 chain structure was present together with Le^a and Le^b compounds. In the blood group B kidneys, the major B glycolipids were monofucosylated hexa- and octaglycosylceramides, where the former were based on the type 2 carbohydrate chain. The blood group B type 4 chain heptaglycosylceramide was found to be a minor component making up only about 1% of the total blood group B structures.

Keywords: glycolipids, blood groups, human kidney, mass spectrometry, proton NMR spectroscopy

Abbreviations: for blood group glycolipid antigens the short hand designation stands for: blood group—number of sugar residues—type of carbohydrate chain. Thus A-7-4 means a type 4 chain blood group A heptaglycosylceramide. The sugar types are abbreviated for mass spectrometry to Hex for hexose, HexNAc for *N*-acetylhexosamine and dHex for deoxyhexose.

The initial characterization of the major, one- to four-sugar-containing, non-acid glycosphingolipid species in human kidney was established several years ago [1–3] and some years later the major gangliosides, including the first description of a fucoganglioside [4], were described by Rauvala [4, 5]. Analysis of single human kidneys by immunostaining of thin-layer plates with monoclonal anti-A antibodies has revealed a complex pattern of blood group A glycolipids and the expression was shown to be dependent on blood group A_1/A_2 and secretor phenotypes of the individuals [6, 7]. These studies are part of a project concerning the characterization of the humoral immune response in ABO incompatible kidney transplantation [6, 7]. In order to obtain sufficient amounts of substance for structural work on the blood group glycolipids identified by monoclonal antibodies, kidneys from several individuals were collected. The initial work revealed the presence of a novel blood group A glycolipid (A-7-4) having the A trisaccharide linked β1-3 to the *N*-acetylgalactosamine of globotetraosylceramide [8]. This compound was the major blood group A glycolipid in kidneys from blood group A individuals. Trace amounts of the Forssman pentaglycosylceramide were also identified [9]. The present paper completes the structural characterization, performed in our laboratory during the last few years, of the major complex blood group type glycolipids in kidneys from blood group A and B individuals.

Materials and methods

Kidney tissue

Human kidneys were obtained from blood group A and B individuals at autopsy. The tissue was cut into pieces and lyophilized. Two batches of blood group A kidneys (520 g and 830 g dry tissue weight) and one batch of B kidneys (116 g dry tissue weight) were analysed.

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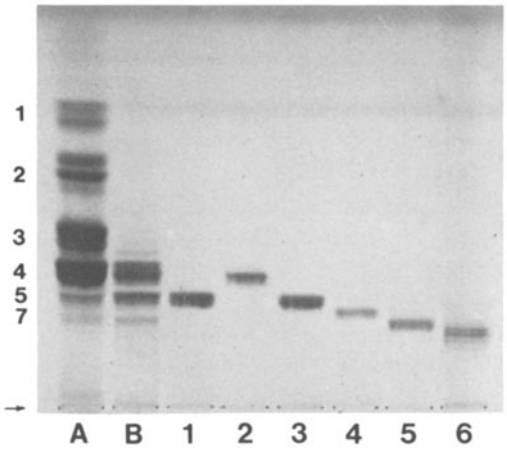

Figure 1. Thin layer chromatogram of total non-acid glycolipids isolated from kidneys of blood group A human individuals (lane A). Lane B shows a polar glycolipid fraction obtained from the total fraction by repeated column chromatography. This fraction was further separated by HPLC into several partly purified fractions (see the Materials and methods section) and the 5- and 7-sugar containing compounds obtained (see lane B) are applied to lanes 1 and 5, respectively. The fraction in lane 1 was treated by α-L-fucosidase and a 4-sugar compound (lane 2) and the remaining 5-sugar compound (lane 3) obtained were separated. The fractions applied in lanes 4 and 6 contain hexa- and hepta/nonaglycosylceramides, respectively. The solvent was chloroform:methanol:water, 60:35:8 by vol. The numbers to the left indicate the approximate number of sugar residues in the compounds of each glycolipid band. The arrow indicates sample origin.

Glycolipid preparation

Total non-acid glycolipid fractions were prepared from the freeze-dried tissue as described in [10]. The amount of glycolipids obtained corresponded to 6.1 mg and 5.9 mg per g dry tissue weight for the blood group A and B kidneys, respectively.

The glycolipid fraction from the first batch of blood group A kidneys (Fig. 1, lane A) was subjected to repeated silicic acid column chromatography and the major part of the glycolipid species with 1–4 sugar residues was removed. The polar glycolipid fraction containing the blood group type compounds (212 mg, Fig. 1, lane B) was further separated into 8 partly purified fractions by gradient elution on an Iatrobeads (Iatron, Japan) column eluted by a continuous gradient of chloroform:methanol:water (from 65:25:4 to 50:40:10, by vol, followed by a final elution with 40:40:12). From the fractions obtained (Fig. 1 in [12]) the Forssman [9], A-7-4 [8] and the globopentaosylceramide/X-5 compounds (described in this paper) were structurally characterized. The second batch of glycolipids from the blood group A kidneys was separated as acetylated derivatives [10, 11] on an Iatrobeads column eluted by a continuous gradient of methanol in chloroform (from 0% to 6%). The fractions obtained were combined according to their TLC mobility as acetylated derivatives, and selected fractions were further purified by repeated Iatrobeads column chromatography. The fractions were deacetylated [10, 11] and the fractions containing blood group type glycolipids were further purified by HPLC using a silica gel column (Polygosil, 5 μm particles, Skandinaviska Genetec, Sweden) and a gradient of isopropanol:hexane:water (from 55:30:10 to 65:15:20, by vol). The hexa- and hepta-nonaglycosylceramide fractions described below and several other minor components were isolated from this preparation.

The blood group B kidney glycolipid fraction was separated as native structures and freed from the majority of glycolipids with 1–4 sugar residues by repeated silicic acid column chromatography. The polar glycolipid fraction obtained (108 mg) was separated by HPLC on a silica gel (see above) column using a solvent gradient of chloroform: methanol:water (from 80:20:1 to 40:40:12, by vol). The fractions obtained were combined according to TLC mobility.

Glycolipid analysis

TLC was performed on HPTLC plates (Merck, Darmstadt, Germany), eluted with chloroform:methanol:water, 60:35:8 by vol. The detection was accomplished by a chemical reagent, anisaldehyde [10], or by autoradiography after staining the plate with various monoclonal anti-blood group ABH antibodies, anti-Forssman antibodies or *Escherichia coli* bacteria, using a modification of the method of Magnani *et al.* [13, 14].

Proton NMR spectroscopy of the intact native glycolipids was performed on a Bruker WH270 (270 MHz) or a Varian XL400 (400 MHz) spectrometer. The samples were dissolved in 0.5 ml [$^2H_6$]dimethyl sulfoxide:^2H_2O (98:2, by vol). Chemical shifts are given relative to tetramethylsilane. In some cases, analysis of the permethylated or permethylated-reduced glycolipid derivatives was performed as described [15]. The glycolipids were permethylated [16, 17] and reduced with $LiAlH_4$ [18] as described.

Mass spectrometry of the permethylated and permethylated-reduced glycolipid derivatives was performed on a ZAB 2F instrument (VG Analytical, Manchester, UK) [19, 20].

The permethylated and/or permethylated-reduced glycolipids were hydrolysed, reduced and acetylated [21, 22] and the partially methylated alditol acetates were analysed by gas chromatography/mass spectrometry on a Trio-2 apparatus (VG Masslab, Manchester, UK). Defucosylation was accomplished using α-L-fucosidase from bovine kidney (Boehringer Mannheim, Germany) [22].

Monoclonal antibodies and E. coli *bacteria*

The mouse monoclonal anti-A (code A581) and anti-B (A582) antibodies as well as the rabbit anti-mouse (Z109) antibody were from Dakopatts a/s (Denmark). Mouse monoclonal antibody KB-26.5 reacting specifically with blood group A type 3 and 4 chain structures [23] was kindly

provided by J. Viñas, Barcelona, Spain. The anti-X antibody $D_1$56-22 [14] and anti-B antibody E_2-83-52 [14] were kindly provided by J. Thurin and H. Koprowski, Wistar Institute, Philadelphia, PA, USA. The anti-Y antibody AH-6 [24] was kindly provided by H. Clausen and S. Hakomori, Seattle, WA, USA, and the anti-Le^a and anti-Le^b antibodies were from Chembiomed Ltd., Edmonton, Canada. *E. coli* bacteria recognizing the Galα1-4Gal sequence [25] were kindly provided by N. Strömberg and K.-A. Karlsson. Radiolabelling of the anti-mouse antibody was performed with $Na^{125}I$ (Amersham, UK) using the Iodo-Gen method (Pierce Chemical Co, Rockford, IL, USA) as described [26].

Results

The HPLC separation of the non-acid glycolipid fractions from the blood group A and B kidneys revealed a very complex pattern of glycolipid species. The fractions were combined according to their TLC mobility, taking into consideration that enough material was present in each fraction to perform the structural studies. The major blood group type glycolipids isolated from the blood group A kidneys are shown in Fig. 1 and selected experimental data are shown in Figs 2–8 and described below. For the blood group B kidneys, the glycolipid fractions obtained are shown in Fig. 9a and the result from the structural work is briefly described (Fig. 9b–d and Fig. 10).

Blood group A kidneys

Pentaglycosylceramide fraction: 27 mg of the pentaglycosylceramide fraction (Fig. 1, lane 1) were isolated from 520 g dry kidney tissue. After acetylation [10] it migrated as a single band on thin layer chromatography. Mass spectrometry of the permethylated and permethylated-reduced derivatives (not shown) revealed a mixture of two components. The major one had the structure Hex-HexNAc-Hex-Hex-Hex-Cer and the minor one dHex-(Hex-)HexNAc-Hex-Hex-Cer. The 270 MHz proton NMR spectrum of the permethylated-reduced fraction (not shown) showed a distinct doublet signal, with a small coupling constant at 5.78 ppm. This signal is specific for a fucose residue linked α1-3 to the *N*-acetylglucosamine of the type 2 chain, as found in the X and Y blood group antigens [15]. Remaining signals in the NMR spectrum were the ones expected for a mixture of a globotetraosylceramide extended with a terminal βGal residue and a blood group X pentaglycosylceramide. This pentaglycosylceramide fraction reacted with the monoclonal anti-X antibody $D_1$56-22 in immunostaining experiments [12]. Treatment of the pentaglycosylceramide fraction (Fig. 1, lane 1) with α-L-fucosidase resulted in a mixture of a 4-sugar compound and a remaining 5-sugar compound which were separated by HPLC. The fractions obtained are shown in Fig. 1, lanes 2 and 3, respectively.

The downfield region of the proton NMR spectrum of the native 4-sugar compound is shown in Fig. 2A. Four β-anomeric resonances ($J_{1,2}$ = 6.6–7.8 Hz) were found at 4.69, 4.27, 4.22 and 4.17 ppm, respectively. The spectrum is identical to that of a reference sample of neolactotetraosylceramide (not shown) and the proton chemical shifts are in accordance with those reported for neolactotetraosylceramide by Dabrowski *et al.* [27].

The mass spectrum of the permethylated-reduced pentaglycosylceramide fraction (Fig. 1, lane 3) isolated after α-L-fucosidase treatment is shown in Fig. 3. The series of peaks at *m/z* 1360–1472 are due to immonium ions containing the whole saccharide chain and non-hydroxy 16:0–24:0 fatty acids. The corresponding fragment for the 24:0 hydroxy fatty acid is seen at *m/z* 1502. Primary carbohydrate sequence ions are found at *m/z* 187 (219 − 32), 219, 450, 654, and 1063. Secondary sequence ions containing the fatty acid and the inner 1–4 sugar residues are seen at *m/z* 615 (614 + 1), 819 (818 + 1), 1023 (1022 + 1), and 1254 (1253 + 1) for the 24:0 fatty acid species as indicated in the formula. In the mass spectrum of the permethylated derivative (not shown) primary carbohydrate sequence ions were present at *m/z* 187, 219 (terminal Hex-), 464 (Hex-HexNAc-), 668 (Hex-HexNAc-Hex-), 873 (Hex-HexNAc-Hex-Hex-), and 1150 (complete saccharide chain plus part of the ceramide). An intense peak at *m/z* 228 due to a non-substituted internal hexosamine was also present. The mass spectra also revealed the presence of trace amounts of other glycolipids as seen for example by peaks at *m/z* 1618 and 1646 in Fig. 3, which are due to the immonium ions for hexaglycosylceramides (2dHex, HexNAc, 3Hex, non-hydroxy 22:0–24:0 fatty acids and/or dHex, HexNAc, 4Hex, hydroxy 22:0–24:0 fatty acids). No sequence ions from these compounds are found in the lower mass region, due to the small amounts present. The carbohydrate sequence ions originating from the Hex-(dHex-)HexNAc-Hex-Hex-Cer compound found in the spectra of the sample before fucosidase treatment (Fig. 1, lane 1 and text above) have all disappeared.

The downfield region of the proton NMR spectrum of the defucosylated pentaglycosylceramide fraction (Fig. 1, lane 3) is reproduced in Fig. 2(b). One α-anomeric signal ($J_{1,2}$ = 3.8 Hz) at 4.82 ppm and four β-anomeric signals ($J_{1,2}$ = 7.2–7.9 Hz) at 4.63, 4.27, 4.21 and 4.17 ppm were present in the spectrum. This spectrum is nearly identical to that obtained for a human teratocarcinoma glycolipid with a Galβ1-3 residue linked terminal to globotetraosylceramide [28] and the interpretation is based on that paper [28] and the data given by Dabrowski *et al.* for the globoseries glycolipids [29].

The major degradation products obtained from the permethylated defucosylated pentaglycosylceramide fractions were identified by gas chromatography/mass spectrometry (Fig. 4a) as 2,3,4,6-*O*-Me_4-Gal (terminal galactose), 2,3,6-*O*-Me_3-Gal (-4Gal1-), 2,3,6-*O*-Me_3-Gal (-3Gal1-) and 4,6-*O*-Me_2-GalNAc (-3GalNAc1-). In addition, some minor components were also found as indicated in the chromatogram.

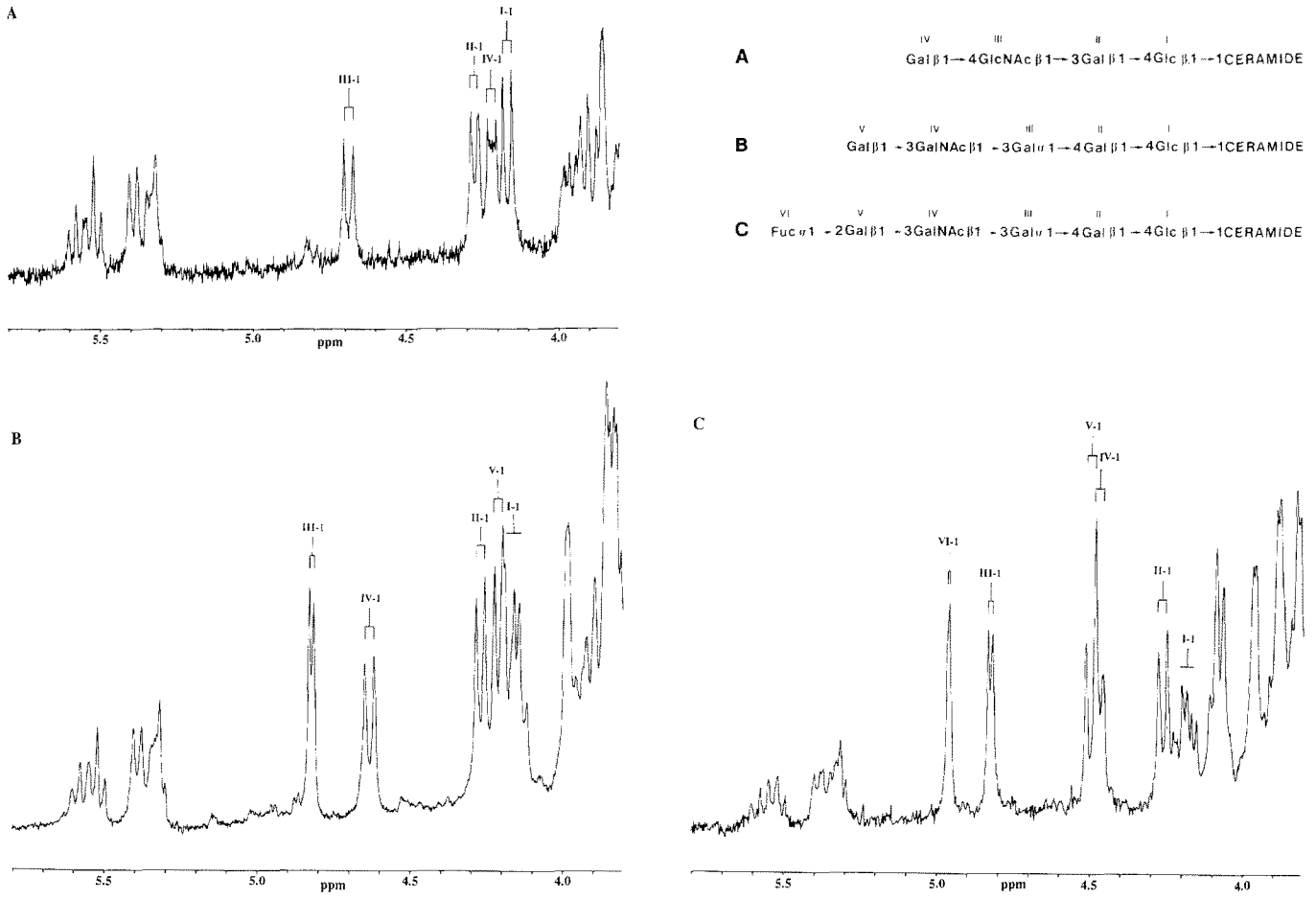

Figure 2. Anomeric regions of the 400 MHz, proton NMR spectra of (a) the tetraglycosylceramide and (b) pentaglycosylceramide compounds isolated after defucosylation of the crude pentaglycosylceramide fraction from blood group A human kidneys (Fig. 1, lanes 2 and 3, respectively). Spectrum (c) shows the hexaglycosylceramide fraction (Fig. 1, lane 4). 450 scans (a), 650 scans (b), and 1163 scans (c) were recorded at 50°C from 1.5 mg (a), 5.0 mg (b), and 3.7 mg (c) of sample, respectively.

In conclusion, the pentaglycosylceramide fraction of the human kidney (Fig. 1, lane 1) consists of a mixture of an extended globoseries compound with the structure Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1Cer (about 80%) and the blood group X pentaglycosylceramide Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glcβ1-1Cer (20%).

Hexaglycosylceramide fraction: A glycolipid fraction was isolated from the blood group A kidneys which in its acetylated form migrated with a TLC R_F value similar to acetylated globotriaosylceramide. After deacetylation, the native compound migrated as a hexaglycosylceramide and the fraction was purified by HPLC into 3 fractions with a slight difference in R_F values. The major one (3.7 mg), used for structural analysis, is shown in Fig. 1, lane 4.

The mass spectrum of the permethylated-reduced derivative is shown in Fig. 5. The intense ions at *m/z* 1618, 1632 and 1646 are due to one dHex, one HexNAc, and four Hex in the saccharide chain and 22:0, 23:0, and 24:0 non-hydroxy fatty acids as indicated in the formula. The peaks at *m/z* 1662 and 1676 are the corresponding fragments with the 23:0 and 24:0 hydroxy fatty acid species. Only trace amounts of short chain (16:0–18:0) fatty acid species were present in this fraction. Carbohydrate sequence ions are found at *m/z* 157 (189−32), 189, 361 (393−32), 393, 625, 828, 1033 and 1237. Molecular ions are seen at *m/z* 1930 (t18:0–24:0) and 1960 (t18:0–h24:0). Rearrangement ions are found at *m/z* 1151 (22:0 non-hydroxy fatty acid) to *m/z* 1209 (24:0 hydroxy fatty acid) due to fragments containing the fatty acid, the three inner hexoses and part of the hexosamine [30], indicating a substitution at the C3 position of the hexosamine similar to the corresponding fragments for type 1 chain structures [30, 31]. In the mass spectrum of the permethylated derivative (not shown), primary carbohydrate sequence ions were found at *m/z* 157, 189 (terminal dHex), 361, 393 (dHex-Hex-), 606, 638 (dHex-Hex-HexNAc-), and 842 (dHex-Hex-HexNAc-Hex-). An intense peak at *m/z* 228 was due to a non-substituted internal hexosamine. The spectra of the two derivatives were similar to those obtained for the blood group H hexaglycosylceramide based on globotetraosylceramide isolated from human meconium [30].

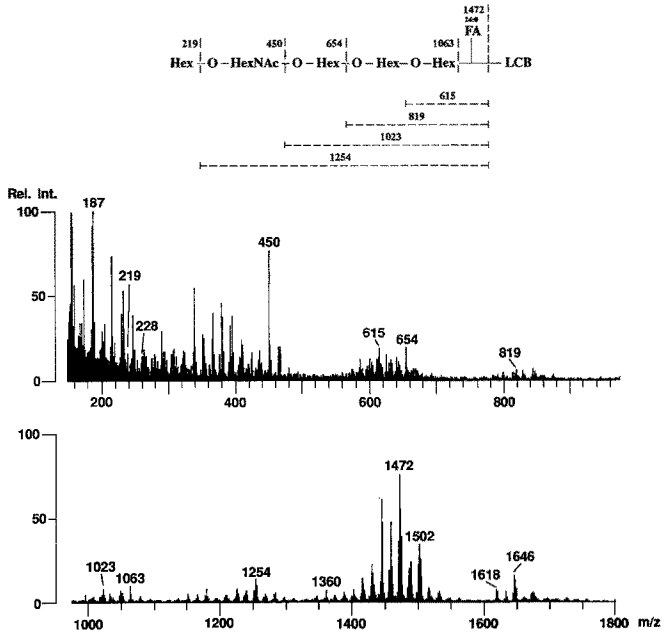

Figure 3. Mass spectrum and simplified formula of the permethylated-reduced pentaglycosylceramide fraction isolated after defucosylation (Fig. 1, lane 3). Conditions of analysis: 10 μg sample; ion source temperature, 290°C; acceleration voltage, 8 kV; trap current, 500 μA; electron energy, 40 eV.

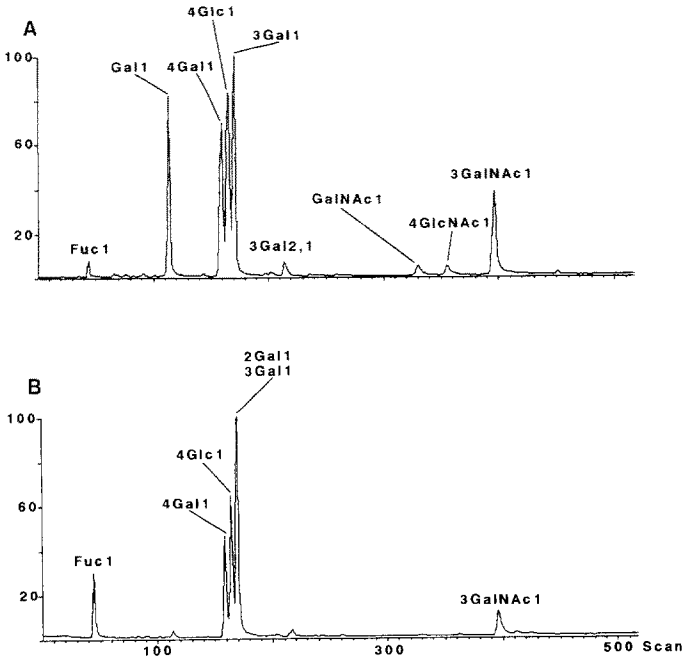

Figure 4. Total ion current curves from the gas chromatographic/mass spectrometric analysis of the products obtained by degradation of (a) the permethylated pentaglycosylceramide fraction obtained after defucosylation (Fig. 1, lane 3), and (b) the permethylated hexaglycosylceramide fraction (Fig. 1, lane 4). The identity of the partially *O*-methylated hexitol and hexosaminitol acetates obtained are given above each peak.

The anomeric region of the proton NMR spectrum of the hexaglycosylceramide fraction (Fig. 2c) revealed a Fucα1-2 signal at 4.96 ppm ($J_{1,2} < 2$ Hz), a Galα1-4 signal at 4.82 ppm ($J_{1,2} = 3.7$ Hz), and 4 β-signals ($J_{1,2} = 6.5$–8.6 Hz) at 4.50, 4.47, 4.26, and 4.18 ppm, as indicated in Fig. 2c. These signals are identical to those reported for a Fucα1-2Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1Cer structure identified in human teratocarcinoma cells [28] and human erythrocytes [32].

Degradation studies of the permethylated hexaglycosylceramide fraction showed the presence of 2,3,4-*O*-Me_3-Fuc (terminal fucose), 2,3,6-*O*-Me_3-Gal (-4Gal1-), 2,3,6-*O*-Me_3-Glc (-4Glc1-), 3,4,6-*O*-Me_3-Gal (-2Gal1-), 2,4,6-*O*-Me_3-Gal (-3Gal1-) and 4,6-*O*-Me_2-GalNAc (-3GalNAc1-) as can be seen in Fig. 4b. From the structural data obtained for the hexaglycosylceramide fraction the following structure was concluded: Fucα1-2Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1Cer. In addition to this compound, several other 6-sugar compounds were present in the kidney (see below) but the large change in R_F value upon acetylation [30] made it possible to obtain this structure virtually free from contaminating glycolipids.

Heptaglycosylceramide fraction: The major 7-sugar glycolipid (Fig. 1, lane 5) was shown to be a globo-series based blood group A compound with the structure GalNAcα1-3(Fucα1-2)Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1Cer, as reported in a separate paper [8].

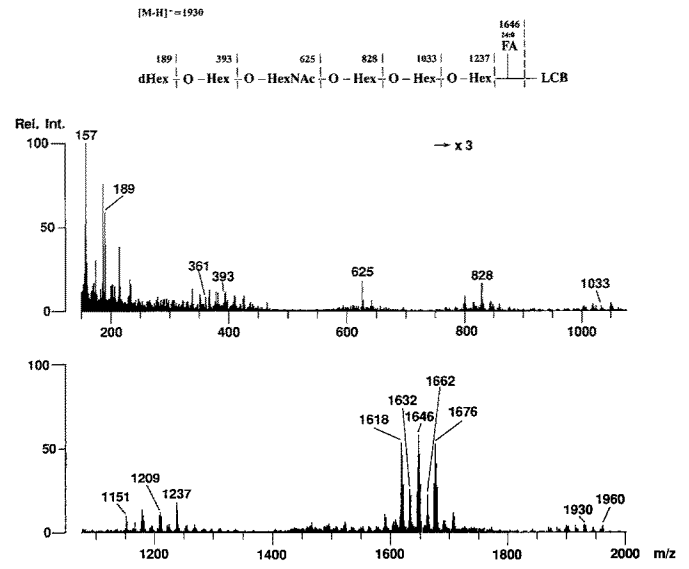

Figure 5. Mass spectrum and simplified formula of the permethylated-reduced hexaglycosylceramide fraction isolated from human blood group A kidneys (Fig. 1, lane 4). Conditions of analysis: 10 μg sample; ion source temperature, 310°C; acceleration voltage, 8 kV; trap current, 500 μA; electron energy, 40 eV.

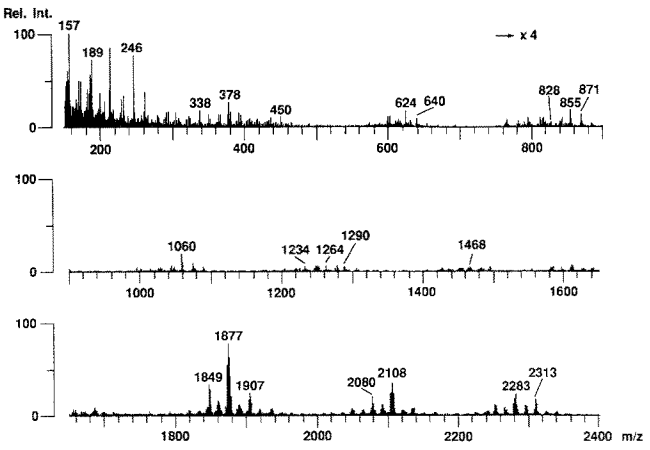

Figure 6. Mass spectrum of the permethylated-reduced hepta/nonaglycosylceramide fraction (Fig. 1, lane 6) isolated from human blood group A kidneys. Conditions of analysis: 16 µg sample; ion source temperature, 360°C; acceleration voltage, 8 kV; trap current, 500 µA; electron energy, 40 eV. Structural formulae for interpretation are given in Fig. 8.

Hepta-/nonaglycosylceramide fraction: 200 µg of a glycolipid fraction migrating as a double band in the 7/9 sugar interval was isolated (Fig. 1, lane 6). This double band was stained strongly in the chromatogram binding assays with the KB 26.5 antibody which reacts specifically with blood group A type 3 and 4 chain structures [23]. The formulae of the four major components deduced from the mass spectrum of the permethylated-reduced derivative (Fig. 6) and proton NMR spectroscopy of the native fraction (Fig. 7) are shown in Fig. 8. Interpretation of the mass spectrum is based on the earlier published spectra of the A-7-4 [8], A-8-2 [33] and A-9-3 [33, 34] compounds and the interpretation of the proton NMR spectrum on the data published by Clausen *et al.* [35–38].

Other minor glycolipid components: In addition to the above described compounds small amounts of several other glycolipids such as the Forssman pentaglycosylceramide [9], the A type 2 hexaglycosylceramide, Le^a penta- and Le^b hexaglycosylceramides were present in the blood group A kidneys.

Blood group B kidneys

The polar non-acid glycolipid fraction (Fig. 9, lane 8) isolated from the blood group B kidneys by silicic acid chromatography was further fractionated by HPLC column chromatography as native derivatives into several partly purified fractions (Fig. 9, lanes 1–20). These fractions were analysed by immunostaining of the thin layer plates with various biological reagents and by mass spectrometry of the permethylated derivatives. Based on the results obtained, selected fractions were further analysed by proton NMR spectroscopy and mass spectrometry of the permethylated-reduced derivatives as described above. As in the blood

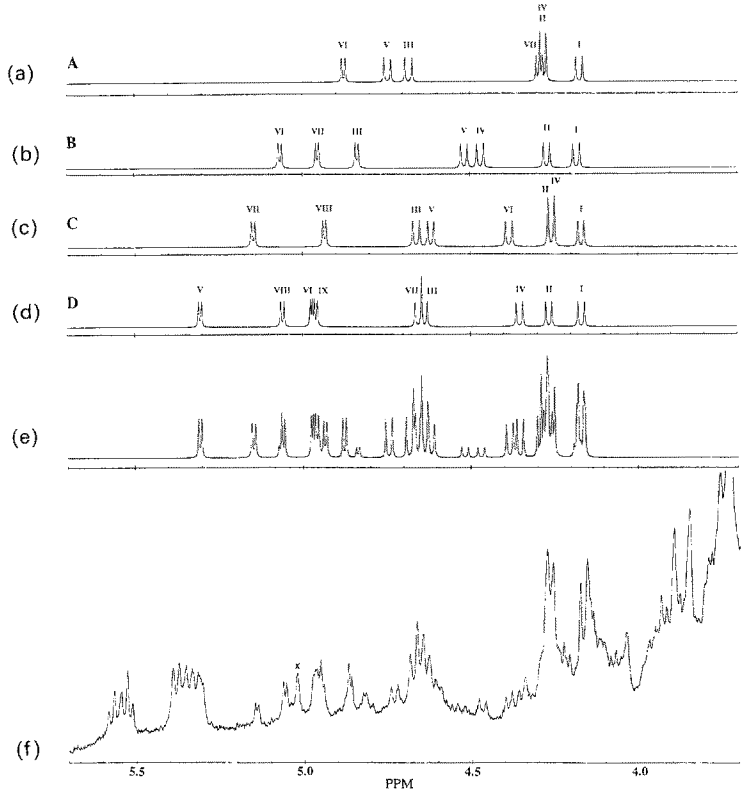

Figure 7. Anomeric region of the 400 MHz proton NMR spectrum of the native hepta/nonaglycosylceramide fraction (Fig. 1, lane 6) isolated from human blood group A kidneys (bottom chart). 100 µg of sample was dissolved in 0.5 ml dimethylsulfoxide-d_6 containing 2% ^2H_2O and analysed at 50°C. 28 400 scans were recorded. (a–d) represent the simulated spectra of the anomeric protons of compounds (a) through (d) in Fig. 8, as reproduced (NMR1 software; New Methods Research, Syracuse, NY, USA) from the chemical shifts and coupling constants of pure reference compounds (b, d) and literature data (compound (a) [35]; compound (c) [38]). In the (e) the simulated spectra are summed together in a ratio of 1:0.3:1:1 (a:b:c:d). The simulated spectra of compounds (b) and (d) were reproduced from spectra recorded at a probe temperature of 50°C and the simulated spectra of compounds (a) and (c) from spectra recorded at 55°C [35] and 35°C [38], respectively. The slight disagreement in the chemical shift of the signal from the proton of residue VIII of compound (c) as compared to the sample spectrum (f) might be due to the difference in probe temperature during acquisition. The signal marked X is due to a non-glycolipid contaminant. Anomeric sugar protons of the structures of Fig. 8 are indicated by their roman numerals.

group A kidneys, the globopentaosylceramide and the blood group X pentaglycosylceramide were the major 5-sugar compounds present in fractions 5 to 10 (Fig. 9). Immunostaining of the plates with two different anti-B antibodies (Fig. 9(c, d)) revealed the presence of blood group B active structures having 6 (fractions 8–11), 8/9 (fractions 13–17), and trace amounts of a structure with 7 sugar residues (fraction 12). The anomeric region of the proton NMR spectrum of fraction 10 is shown in Fig. 10. This spectrum

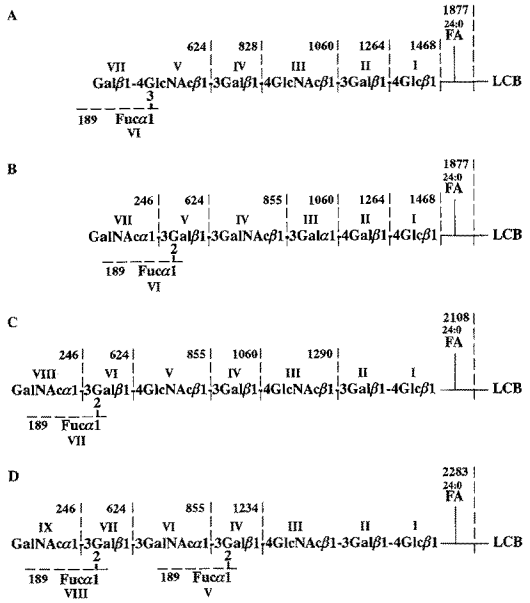

Figure 8. Structural formulae of the four major glycolipid compounds identified by mass spectrometry (Fig. 6) and proton NMR spectroscopy (Fig. 7) of the hepta/nonaglycosylceramide fraction (Fig. 1, lane 6) isolated from human blood group A kidneys. The arabic numbers indicate the mass of the fragments obtained by mass spectrometry of the permethylated-reduced derivative (Fig. 6) and the roman numbers indicate the anomeric sugar protons of the compounds shown in Fig. 7.

shows a complex mixture of components with the blood group H type 4 chain structure as the major glycolipid (c) as indicated in the figure (compare Fig. 2c). A type 2 chain blood group B hexaglycosylceramide (d) is also seen together with small amounts of the globopentaosylceramide (a), the X-5 (b) and the Le^b-6 (e) structures. The presence of these structures was further supported by mass spectrometry (not shown).

Special interest was focused on the identification of a blood group B determinant based on the globotetraosyl core saccharide (B type 4 chain). Staining of the thin-layer plate with *E. coli* known to bind to an internal Galα1-4 sequence [25] revealed (Fig. 9b) staining of the 3 sugar (globotriaosyl-), 4 (globotetraosyl-), 5 (globopentaosyl-), and 6 (H-6-4) sugar regions, but also a weak band migrating just below the A-7-4 reference was found in fractions 12 and 13. This region was also stained by one of the anti-B antibodies (Fig. 9d). Mass spectrometry and proton NMR spectroscopy of fraction 12 gave information consistent with the presence of small amounts of a heptaglycosylceramide with a blood group B terminal and a globotetraosylceramide core structure. Experimental data will be presented in detail elsewhere (Holgersson J, Samuelsson BE, Breimer ME; unpublished results). The amount of the B-7-4 was estimated to be about 1% of the total blood group B glycolipids.

Discussion

The present work is part of a project concerning the expression of blood group ABH and related antigens in the human kidney [6, 7] and the immune response against these antigens in ABO incompatible transfusions [26] and organ transplantations [7, 39]. Studies of single kidneys initially obtained for transplantation purposes but discarded for various reasons have shown an individual specific distribution of blood group A antigens related to the A_1/A_2 and secretor status of the tissue donors [6, 7]. These analyses were performed by immunostaining the total glycolipid fractions on thin layer plates and a highly complex pattern of blood group A antigens in the different kidneys was found [6, 7]. Due to the very limited amount of blood group type glycolipids present in the single kidney fractions, no structural work could be performed on these cases. In order to obtain sufficient material for a structural characterization of the blood group type glycolipids, kidneys from several tissue donors were collected at autopsy and analysed. Previous work by Mårtensson [2, 3] and Makita [1] on the structure of human kidney glycolipids were, due to the analytical limitations at that time, restricted to the simple compounds with up to 4 sugar residues with globotriaosylceramide and globotetraosylceramide as the major compounds. The present study revealed a very complex mixture of blood group type glycolipids and compounds with up to 9 sugar residues were structurally characterized. The presence of several components made the purification of single components difficult and, in several cases, the analysis of minor components had to be performed on glycolipid mixtures. The kidneys analysed were obtained at autopsy and therefore contain plasma and blood cells in contrast to the single kidneys analysed by the monoclonal antibody overlay technique [6, 7], where the kidneys were perfused with kidney storage solution. Some of the minor components present in the autopsy kidney fractions may therefore not originate from the kidney tissue itself, why the characterization of very minor components may be of limited value.

For human tissues, the kidney is unique in the sense that the major parts of glycolipids with more than 4 sugar residues are extended globo-series compounds. The Forssman pentaglycosylceramide was for a long time the only glycolipid identified having an extension of the globotetraosyl saccharide chain. It was present in trace amounts in human tissues such as gastric tissue [40] and kidneys [9], in contrast to the amounts found in species such as goat, horse and mouse, where it is a major component [41]. The globopentaosylceramide has not been found before in any other adult human tissue, although it was present in a human teratocarcinoma cell line [28]. The blood group H glycolipid based on globotetraosylceramide was first identified in human meconium [30] and has now been found in a human teratocarcinoma cell line [28], human erythrocytes [32] and kidney (this paper). The corresponding blood

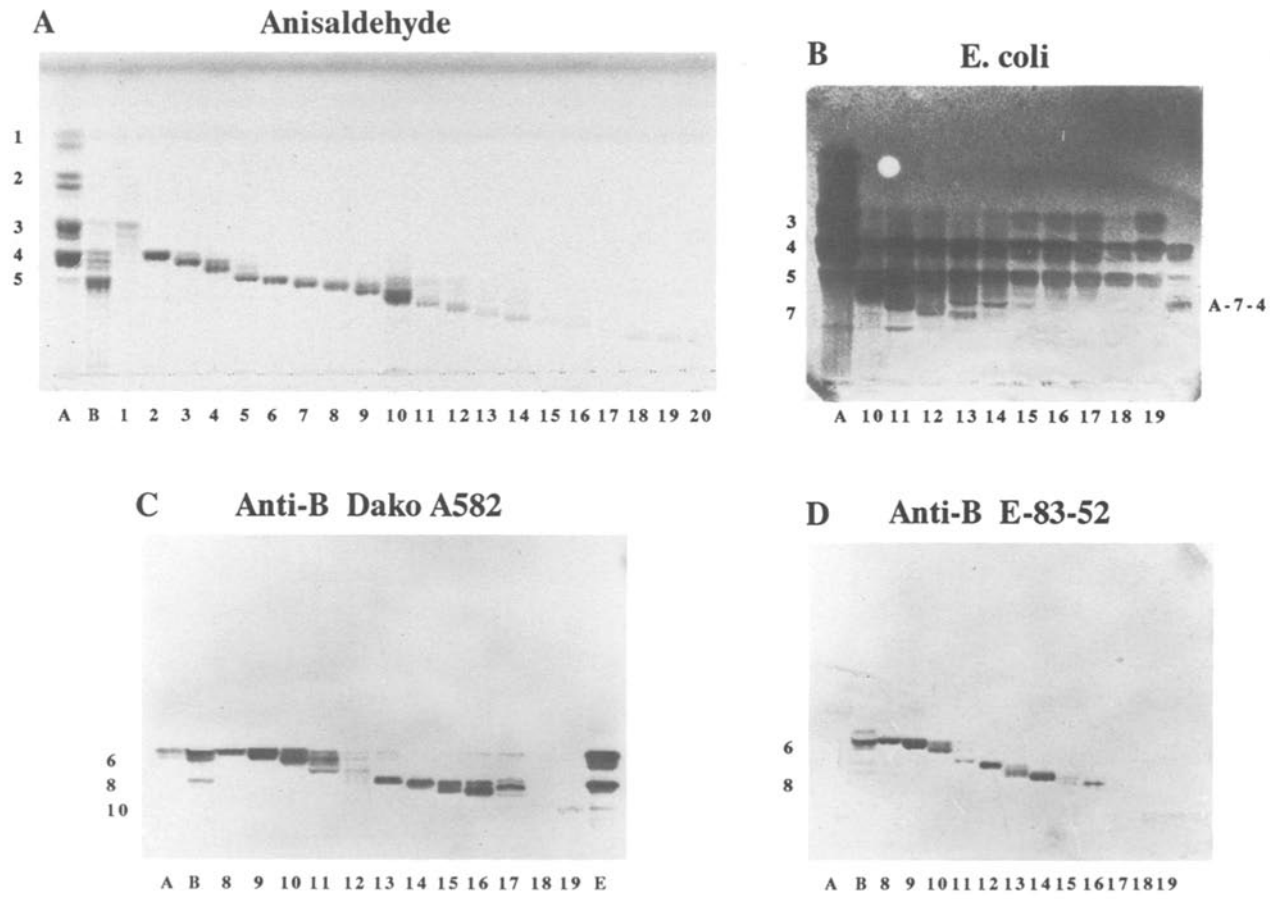

Figure 9. Thin layer chromatograms of total non-acid glycolipids isolated from kidneys of blood group B human individuals (lane A). Lane B shows a polar glycolipid fraction obtained from the total fraction by repeated column chromatography and this fraction was further separated by HPLC chromatography (lanes 1 to 20). Detection was accomplished by (a) the chemical reagent anisaldehyde, (b) *E. coli* bacteria, or (c, d) by probing the plates with two different monoclonal anti-B antibodies. The solvent was chloroform:methanol: water, 60:35:8 by vol.

group A heptaglycosylceramide has been found in trace amounts in human erythrocytes [35] while it is the major blood group A glycolipid in the human kidney [8]. Also sialic acid [28, 42] and sulfate [43] containing globoseries glycolipids have been found.

The high levels of extended globoseries compounds is also of interest in relation to the studies showing that uropathogenic *E. coli* bacteria bind selectively to the Galα1-4Gal sequence of the globoseries glycolipids [25, 44, 45]. Further detailed studies have shown that certain *E. coli* strains selectively prefer the Globo-A carbohydrate structure as their receptor [46, 47]. This component is a major blood group component of the ureter epithelium [48]. However the role for globoseries glycolipids in the pathogenesis of urinary tract infection still remains to be elucidated.

Blood group ABH antigens in human tissues have been classified as type 1 (-Galβ1-3GlcNAc-), type 2 (-Galβ1-4GlcNAc-), type 3 (-Galβ1-3GalNAcα1-, "repetitive A") and type 4 (-Galβ1-3GalNAcβ1-, "globo-A") according to the core saccharide type. In the blood group A kidneys all these types of A structures have been identified [present work, 6–8] while in the blood group B kidneys, blood group B hexa- and octaglycosylceramides (type 1/2) were the dominating ones and only trace amounts of B type 4 antigen was found. The expression of the A type 4 antigen in the kidney has been shown to be dependent on the secretor gene [6] and, since the Le^b antigen was found in the blood group B kidneys, several of the kidney donors must be secretor individuals. The expression of A type 4 chain antigens in human erythrocytes was shown to be dependent on the A_1/A_2 gene status of the donors [49] where A_2 individuals lacked or contained only trace amounts of the A type 4 antigen. A similar expression was also noted for A kidneys of single individuals [6]. Preliminary enzymatic and immunochemical studies by Clausen and Hakomori [49] and by ourselves (Holgersson J, Samuelson BE, Breimer ME; unpublished results) indicate that the B transferase is almost incapable of adding a galactose residue to the type 4 chain H substrate. Therefore, the blood group B galactosyltransferase seem to be similar to the blood group

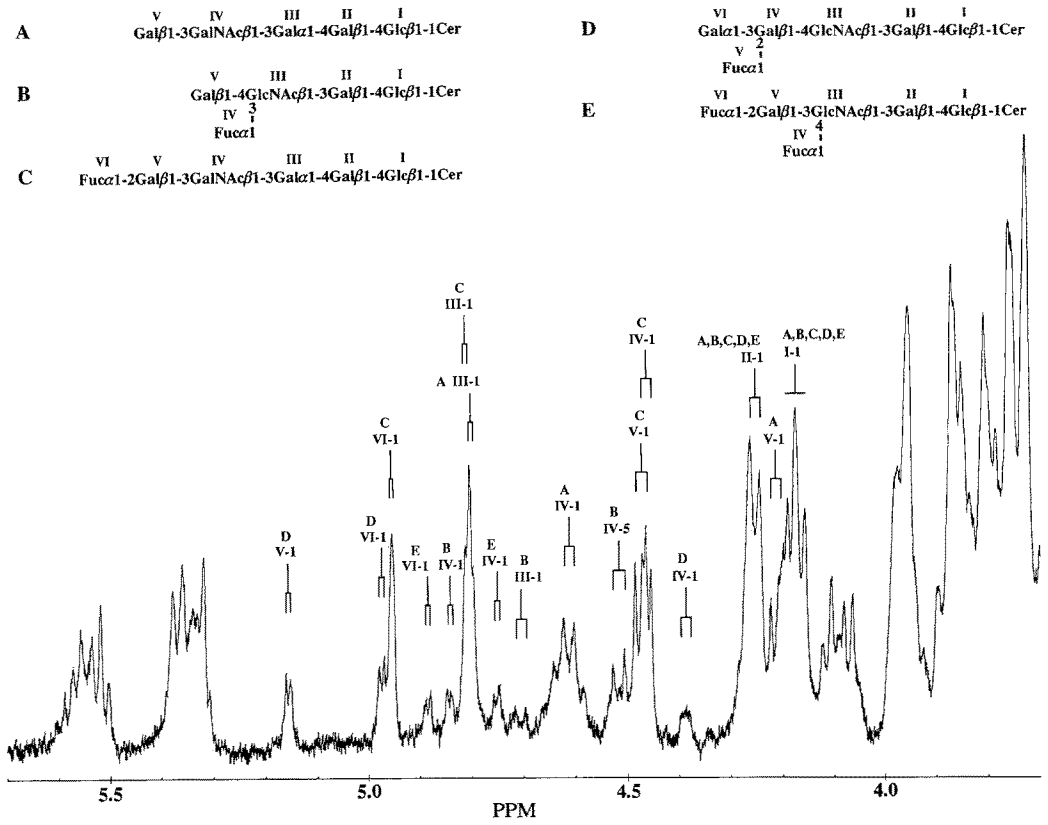

Figure 10. Anomeric region of the 400 MHz proton NMR spectrum of the glycolipid fraction number 10 (Fig. 9) isolated from human blood group B kidneys. 600 μg sample was dissolved in 0.5 ml [$^2H_6$]dimethyl sulfoxide containing 2% ^2H_2O and analysed at 30°C. 2220 scans were recorded. The proton signals are indicated by a letter corresponding to the structure (as shown by the formulae), a roman numeral corresponding to the sugar residue, and an arabic numeral indicating the position in the pyranose ring (1 = anomeric proton).

A_2 *N*-acetylgalactosaminyltransferase in its low acceptance of the type 4 chain H precursor glycolipid.

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