Blood group H antigen with globo-series structure

Isolation and characterization from human blood group O erythrocytes

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Blood group H antigen with globo-series structure, reacting with the monoclonal antibody MBrl, was isolated and characterized from human blood group O erythrocytes. The structure was identified by methylation analysis, direct probe mass spectrometry, and ^{1}H-nuclear magnetic resonance spectroscopy as shown below: Fucα1 → 2Galβ1 → 3GalNAcβ1 → 3Galα1 → 4Galβ1 → 4Glcβ1 → 1Cer

Blood group H antigen *Globo-series structure*

1. INTRODUCTION

Blood group ABH determinants in human erythrocytes are carried by type 2 chain in either glycolipids or glycoproteins [1]. Recently, a series of extended globo-series glycolipids has been isolated and characterized from human teratocarcinoma 2102. Among these, a new type of blood group H antigen with globo-series structure was characterized and designated 'Gl-6' [2]. In human adult cells and tissues, the presence of extended globo-series structures has been unknown, except for the presence of a small quantity of Forssman glycolipid in tissues of some human populations (Fs^+ group) and in some human cancers [3], and the presence of 'para-Forssman' glycolipid in human erythrocytes [4]. Recently, a monoclonal antibody (MBrl) directed to breast cancer and normal mammary gland epithelia was shown to react specifically with 'GL-6', the globo-series H antigen [5]. During a systematic fractionation of blood group active glycolipids in human erythrocyte membranes, we noticed the presence of a blood group H glycolipid with globo-series structure (hereby designated 'globo-H') reacting with antibody MBrl. We report the isolation and characterization of this new type of blood group H antigen from human erythrocytes.

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Abbrevations: HPLC, high performance liquid chromatography; HPTLC, high performance thin-layer chromatography; NMR, nuclear magnetic resonance. Glycolipids are abbreviated according to the recommendations of the Nomenclature Committee, IUPAC [13], but the suffix 'OseCer' is omitted

2. MATERIALS AND METHODS

Human erythrocyte membranes were extracted with isopropanol–hexane–water (55:25:20, lower phase) and the upper neutral glycolipid fraction was prepared from the total extract as previously described [6]. The fraction containing x-series glycolipids (x_1–x_4, eluted between H_1 and H_2) [7] was prepared by low-pressure HPLC on Iatrobeads 6RS-8060 column, and this fraction was rechromatographed on 6RS-8010 column with high-pressure HPLC according to the method originally described in [8] and modified subsequently [6]. The x_3 glycolipid fraction (fig.1) eluted

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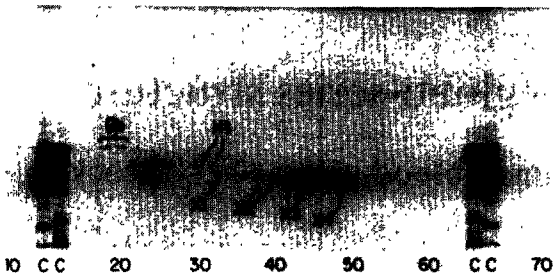

Fig.1. Separation pattern of x-series glycolipids of human blood group O erythrocytes. Separation was performed on Iatrobeads 6RS-8010 column in an isopropanol–hexane–water gradient of 55:40:5 to 55:30:15. The original sample applied to the column was the fraction containing x-series glycolipids eluted from the first low-pressure chromatography as previously described [7]. HPTLC was developed in chloroform–methanol–water (65:35:8).

between tube number 38–48 was pooled and acetylated in pyridine–acetic anhydride (2:1), and further purified on HPTLC plates developed in a solvent mixture of 1,2-dichloroethane–acetone–water (60:40:0.1). Acetylated derivatives of Gb_3, globoside, Gg_4, and nLc_6 were run as controls. Among a few components of the acetylated x_3 fraction, the fastest migrating component, which migrated between acetylated globoside and acetylated Gb_3, was scraped and extracted with chloroform–methanol (2:1). Glycolipid acetates separated on HPTLC were revealed by ultraviolet light after spraying with 0.02% Primulin (Aldrich, Milwaukee, WI) in acetone–water (2:1) [9]. The yield of glycolipid was ~100 μg from 2 l packed erythrocytes. The monoclonal antibody MBrl [10] was a gift from Dr S. Sonnino (Università Degli Studi Di Milano, Italy). The antibody was selected by its specific reactivity to breast cancer, and its epitope structure was identified as globo-H [5]. Immunostaining of glycolipid was performed according to the procedure of [11]. Methylation analysis and direct-probe mass spectrometry were performed as previously described [2,6,7]. Proton NMR spectra were obtained on a Bruker WM-500 spectrometer operating in the Fourier-transform mode and using quadrature detection. A glycolipid sample (100 μg) was deuterium-exchanged and spectra were taken in 0.4 ml $MeSOd_6$ containing 2% D_2O and 1% tetramethylsilane as a chemical shift reference. The procedure is essentially the same as previously described [12].

3. RESULTS

A series of glycolipids eluted from the Iatrobeads column between H_1 and H_2 glycolipids were called x_1, x_2, x_3, and x_4, according to the sequence of elution as shown in fig.1. Two components in the x_3 fraction, x_3a and x_3b, and the x_2 glycolipid were clearly separated on HPTLC as acetates. The fast-migrating component with a mobility between acetylated Gb_3 and acetylated Gg_4 (x_3a, fig.2, lane 1, panel A) and the component with the slowest mobility (x_3b, fig.2, lane 3, panel A) were clearly separated to homogeneity. The acetylated x_2 glycolipid migrated as an intermediate (fig.2, lane 2, panel A) was identified as having the structure GalNAcβ1⟶3Galβ1⟶4GlcNAcβ1⟶3Galβ1⟶4Glcβ1⟶1Cer, in agreement with previous studies [7]. The x_3b

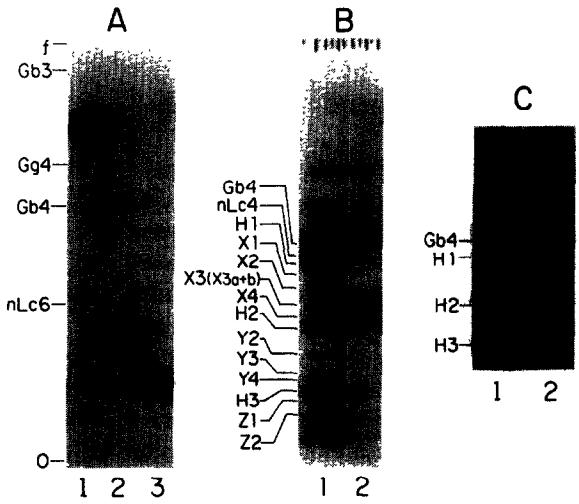

Fig.2. HPTLC pattern of purified x_3a (globo-H) glycolipid. Panel A: Purified glycolipid acetates. Lane 1, x_3a; lane 2, x_2; lane 3, x_3b. The position of acetylated derivatives of each glycolipid is indicated in the left margin. Solvent, see text. Stained by orcinol–sulfuric acid. Panel B: lane 1, upper neutral glycolipid of blood group O erythrocytes; lane 2, purified deacetylated x_3a glycolipid (globo-H antigen). Left margin indicates the position of characterized glycolipids (1,6,7). Note that x_3 component is very minor. Panel C: HPTLC of upper neutral glycolipids of O erythrocytes stained by orcinol–sulfuric acid (lane 1) and its immunostaining pattern by MBrl antibody (lane 2). HPTLC in panels B and C developed with chloroform–methanol–water (60:35:8).

glycolipid was identified as having the structure GalNAcβ1 ⟶ 3GalNAcβ1 ⟶ 3Galα1 ⟶ 4Galβ1⟶4Glcβ1⟶1Cer, which is identical to that previously described [4] and termed 'para-Forssman'. Since the structures of these glycolipids have been previously elaborated, the supporting data for each structure are not presented here. The x_3a glycolipid, after deacetylation, was homogeneous (fig.2, lane 2, panel B) and was stained by the monoclonal antibody MBrl (fig.2, lane 2, panel C). This antibody was recently identified as being directed to globo-series H antigen (Fucα1⟶2Galβ1⟶3GalNAcβ1⟶3Galα1⟶4Galβ1⟶4Glcβ1⟶1Cer) [5].

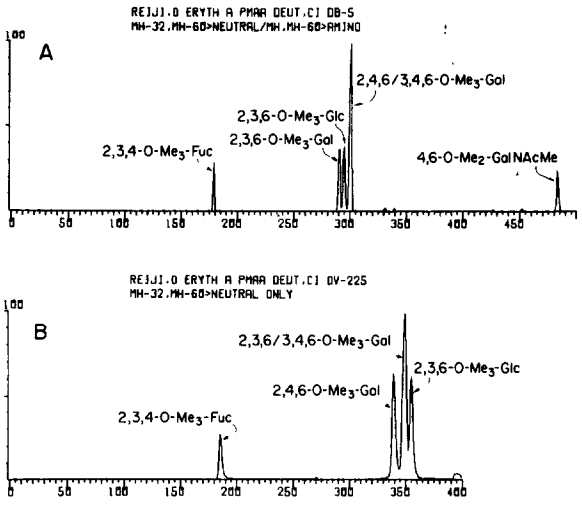

Fig.3. Gas chromatography–mass spectrometry of partially O-methylated hexitol and hexosaminitol acetates of x_3a glycolipid. Separation performed on capillary column DB-5 in panel A and on OV-225 in panel B. Peaks are identified as indicated.

On methylation analysis, the purified x_3a glycolipid gave 2,3,4-tri-O-Me-Fuc, 2,3,6-tri-O-Me-Gal, 2,3,6-tri-O-Me-Glc, and 4,6-di-O-Me-GalNAcMe (fig.3A). 3,4,6-Tri-O-Me-Gal was separated from 2,4,6-tri-O-Me-Gal under different conditions of gas chromatography (capillary column OV225) (fig.3B). On direct probe mass spectrometry, the following fragments were detected: m/z 189 ⟶ 157, 228 (but not 260), 393 ⟶ 361, 638 ⟶ 606, and 843 ⟶ 811 (see fig.4). These fragments agree very well with the sequence indicated in the insert to fig.4. The absence of a fragment at m/z 182 and the presence of the fragment at m/z 228 indicate a preferential loss of Deoxyhex-O-Hex-OH from the 3 position of the internal HexNAc.

The NMR spectrum of this glycolipid showed a characteristic pattern for globo-series H (fig.5) as previously reported [2]. However, resonances for the anomeric protons of the penultimate Gal (V-1 in fig.5) and GalNAc (IV-1 in fig.5) residues were separated at 308 K, although they were not separated in a previous study at 303 K [2]. The triplet at 4104 ppm (308 K) was assigned as H-5 of α-Gal residue (III-5 in fig.5) based on the rationale as previously described [2]. The quartet at 4076 ppm (308 K) was assigned as H-5 of α-Fuc (IV-5 in fig.5) by decoupling from the Fuc methyl doublet (IV-6) at 1087 ppm (J = 6.1 Hz). The assignments for II-4 and III-4 (fig.5) were made tentatively on the basis of comparison with the data of [12] for globoside and Forssman antigen. Details of the assignment of each resonance in NMR spectra of extended globo-series glycolipids will be published elsewhere.

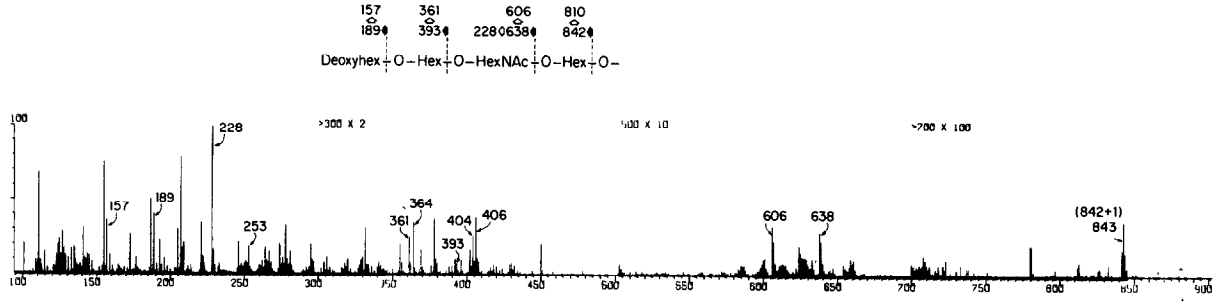

Fig.4. Direct probe mass spectrometry of permethylated x_3a glycolipids in Finnigan 3700 mass spectrometer with 6100 data system under the following conditions: electron energy, 35 V; ion energy programmed from +5.5 V; extractor, +7.7 V; lens, 30 V; emission, +0.5 mA; electron multiplier, 2200 V; and sensitivity, 10–7 AN.

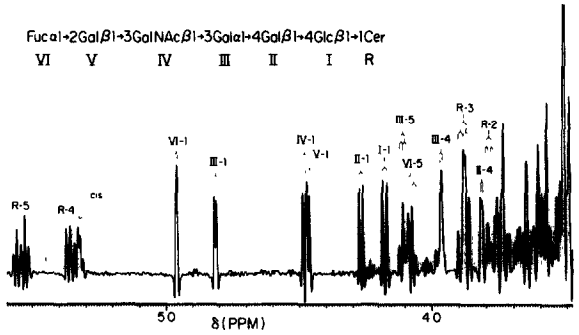

Fig.5. Proton NMR spectrum of x_3a glycolipid. 100 μg of glycolipid with 2000 scans. Probe temperature was 308 K. Sugar residues are indicated by roman numerals and positions of protons are indicated by arabic numbers. For assignment of resonances for each proton, see text.

4. DISCUSSION

Extended globo-series glycolipids have been found as major components of human teratocarcinoma [2] and human breast cancer [5]; however, their presence has not been chemically established in human adult tissues, except for a small quantity of Forssman and 'para-Forssman' glycolipids [3,4]. The monoclonal antibody MBrl, which is directed to a human breast cancer cell line, defines a glycolipid with the structure identical to 'GL-6', previously isolated from human teratocarcinoma [5]. The antigen has now been isolated from human blood group O erythrocytes as one of the x-series glycolipids (x_3a component) eluted between H_1 and H_2. The method of isolation described here is typical for purification of minor components present in a mixture of glycolipids, i.e., two steps of HPLC on Iatrobeads, separation of acetylated fraction on HPTLC, isolation of a single component, followed by deacetylation. The TLC migration of acetylated glycolipids often shows unusual mobility. The globo-series H antigen is a ceramide hexasaccharide, but its acetylated derivative showed a much faster TLC mobility than acetylated ceramide tetrasaccharide (acetylated globoside and acetylated asialo GM_1). Methylation analysis, direct probe mass spectrometry, and NMR spectrometry unambiguously indicate that the purified glycolipid has the structure shown below:

Fucα1 ⟶ 2Galβ1 ⟶ 3GalNAcβ1 ⟶ 3Galα1 ⟶ 4Galβ1 ⟶ 4Glcβ1 ⟶ 1Cer

Two ceramide pentasaccharides showing a much slower mobility than globo-H antigen as acetylated derivatives were isolated, one of which was identified as x_2 glycolipid (GalNAcβ1 ⟶ 3Galβ1 ⟶ 4GlcNAcβ1 ⟶ 3Galβ1 ⟶ 4Glcβ1 ⟶ 1Cer) and the other as 'para-Forssman' (GalNAcβ1 ⟶ 3GalNAcβ1 ⟶ 3Galα1 ⟶ 4Galβ1 ⟶ 4Glcβ1 ⟶ 1Cer). As free compounds, the globo-H antigen and these glycolipids are hardly separated on HPTLC.

This is the first clear chemical demonstration of the existence of globo-series H antigen in normal adult human cells. The possible presence of blood group A or B analogues with globo-series structure is anticipated and is under investigation.

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