BLOOD-GROUP (A + H) FUCOLIPIDS – A NEW GROUP OF COMPLEX GLYCOSPHINGOLIPIDS

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1. Introduction

Studies on blood-group (A+H) glycoprotein antigens of hog gastric mucosa have shown the presence of A and H determinants at the nonreducing terminal ends of the branched carbohydrate chain [1–4]. Such oligosaccharide chains show considerable variance with respect to sugar composition and degree of branching. Similar degree of variance, with respect to sugar composition and complexity of oligosaccharide chains, also have been found in the blood-group fucolipid antigens of hog gastric mucosa [5–8]. However, at present, all of the characterized fucolipids of this tissue carry either A or H determinants [7,9,10].

During the course of studies on complex glycosphingolipids of hog gastric mucosa, obtained by a newly-developed extraction procedure [11,12], a new group of fucolipids bearing (A+H) blood-group determinants was isolated. At least 4 types of (A+H)-active glycolipids, composed of 12 and 14 sugar residues, have been distinguished. We describe here the isolation and some of the characteristics of these compounds.

2. Experimental

2.1. *Materials*

Hog stomachs used for mucosa preparation were purchased from Pel-Freez Biological (Rogers, AR). Human red cells A, B and O types, human blood-grouping serum anti-A, anti-B and anti-H (*Ulex europeus* extract) were obtained from Biol. Corp. Am. (Port Reading, NJ). DEAE–Sephadex A-25 was from Pharmacia (Piscataway, NJ), silicic acid (100–200 mesh) from Bio-Rad (Richmond, CA) and silica gel HR plates from Analtech (Newark, DE).

2.2. *Isolation of glycolipids*

Hog gastric mucosa scrapings obtained from several fresh stomachs were dried with acetone, homogenized with 10 vol. chloroform/methanol (2/1) and filtered. The insoluble residue was then extracted twice, each time with 10 vol. 0.4 M sodium acetate in methanol/chloroform/water (60/30/8), and filtered [11]. The combined filtrate was evaporated to dryness and treated with 0.2 M NaOH in methanol. After 1 h at 37°C, the sample was taken up in an excess of water and dialyzed. The non-diffusible lipids were lyophilized, dissolved in methanol/chloroform/water (60/30/8) and fractionated on a DEAE-Sephadex [11] column (2.5 X 40 cm). The crude fucolipid fraction eluted from the column with 2100 ml above solvent mixture was dried and acetylated with acetic anhydride/pyridine [12]. Lipids recovered from the acetylation mixture by evaporation with toluene were dissolved in 1,2-dichloroethane and chromatographed on a silicic acid column. The acetylated fucolipids, eluted from the column with 1,2-dichloroethane/acetone (1/1) and acetone, were further separated into individual components by thin-layer chromatography in 1,2-dichloroethane/methanol/water (80/25/2) and chloroform/acetone/methanol/water (50/40/20/4).

2.3. *Analytical methods*

Methyl esters of fatty acids and methyl glycosides were obtained by methanolysis of glycolipids [14].

The alditol acetate derivatives of monosaccharides were obtained from the glycolipids according to the procedure in [15]. The long-chain bases were determined by the method in [16]. Thin-layer chromatography was performed on silica gel HR plates activated at 130°C for 1 h. The glycolipids were visualized with orcinol reagent. Visualization with iodine vapors was used for preparative purposes.

Gas–liquid chromatography analyses of fatty acid methyl esters and trimethylsilyl derivatives of methyl glycosides were performed on the columns (180 X 0.2 cm) packed with 3% SE-30 on Chromosorb, W, AW, DMCS [14]. Alditol acetates were analyzed on 1% ECNSS-M columns [14].

Hemagglutination and hemagglutination-inhibition assays were performed with the Takatsy microtitrator using 0.025 ml loops and a 2% suspension of human red cells. The anti-A and anti-B sera were diluted to 4 units; the anti-H was commercially available at potency of 2 units. Isolated fucolipids prior to assay were deacylated with sodium methoxide [17]. The assays were performed with or without a 10-fold excess of auxiliary lipids [14].

3. Results

The lipids extracted from 400 g dry mucosa scrapings with sodium acetate in methanol/chloroform/water, after alkaline methanolysis and DEAE-Sephadex column chromatography, yielded 3.91 g crude neutral glycolipids. The fucolipids present in this fraction were acetylated and resolved into 2 fractions on a silicic acid column. The fraction eluted from the column with 1,2-dichloroethane/acetone contained fucolipids I and II, whereas the acetone eluate contained fucolipids III and IV. A thin-layer chromatogram of the isolated fucolipids is shown in fig.1. The yields of purified glycolipids I, II, III and IV were 4.1 mg, 3.3 mg, 2.9 mg and 3.5 mg, respectively.

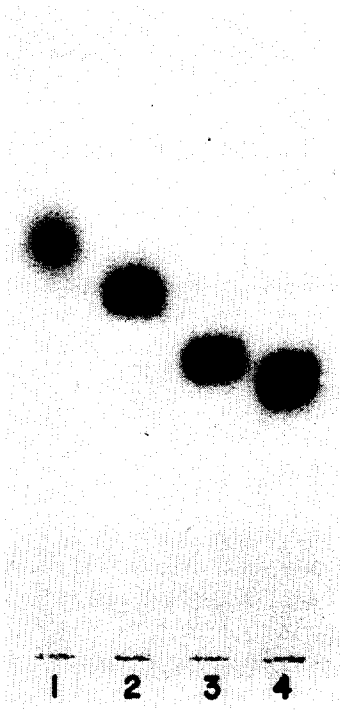

Fig.1. Thin-layer chromatogram of the acetylated blood-group (A+H) fucolipids purified from hog gastric mucosa. (1) Fucolipid I; (2) fucolipid II; (3) fucolipid III; (4) fucolipid IV. Conditions: Silica gel HR 250 nm developed in 1,2-dichloroethane/methanol/water (80/25/2). Visualization: orcinol reagent.

Gas–liquid chromatography of the alditol acetates and methyl glycosides, formed from the carbohydrate

Table 1
Molar ratios of sphingosine and carbohydrates in the isolated fucolipids of hog gastric mucosa

Fucolipid	Molar ratios[a]					
	Fuc	Gal	Glc	GlcNAc	GalNAc	Sphingosine
I	2.08	5.02	1.0	3.12	1.0	0.9
II	1.95	3.98	1.0	3.87	1.01	1.0
III	1.92	5.78	1.0	4.05	0.92	0.9
IV	2.05	4.92	1.0	5.01	0.95	1.1

[a] Relative to Glc = 1

portions of the glycolipids, established the presence of fucose, galactose, glucose, *N*-acetylglucosamine and *N*-acetylgalactosamine. The molar ratios of sugars and sphingosine present in the isolated fucolipids are given in table I. These results indicate that the oligosaccharide chains of fucolipids I and II contained 12 sugar residues, and fucolipids III and IV, 14 sugar residues. Although the fucolipids I and II, and III and IV contained identical numbers of sugar residues, they clearly differed from each other in migration on thin-layer plates (fig.1) and in the number of galactose and *N*-acetylglucosamine residues. Fucolipid I contained 5 galactose and 3 *N*-acetylglucosamine residues, whereas fucolipid II contained 4 galactose and 4 *N*-acetylglucosamine residues. Six galactose and 4 *N*-acetylglucosamine residues were present in fucolipid III, whereas fucolipid IV contained 5 galactose and 5 *N*-acetylglucosamine residues. Apparently, these differences were sufficient to affect the separation of the acetylated compounds during thin-layer chromatography.

In hemagglutination-inhibition assays all 4 fucolipids were potent inhibitors of agglutination of human group A-cells by anti-A serum and human H-cells by anti-H lectin (*Ulex europeus*). The extent of A activity of fucolipids I, II, III and IV against 4 units of anti-A serum was 0.6, 0.8, 1.1 and 1.7 μg/0.1 ml, respectively, and was not significantly affected by the addition of auxiliary lipids. The H activity of fucolipids I, II, III and IV with or without auxiliary lipids, was 0.9, 1.0, 1.2 and 1.9 μg/0.1 ml, respectively, against 2 units of anti-H lectin. Thus, the above data clearly indicate that each of the isolated fucolipids bears 2 blood-group determinants, A and H.

Analyses of the ceramide portion of the fucolipids revealed that shingenine was the major base of all 4 compounds, and hexadecanoate, octadecanoate, octadecenoate and eicosanoate were the principal fatty acids.

4. Discussion

The occurrence of multiple forms of fucolipids with ABH antigenic properties in the gastrointestinal tissue of mammals is well established [18,19]. All of these compounds carry one type of blood-group determinant, which is compatible with the blood type of the host. Thus, the currently known fucolipids of hog gastric mucosa display either A or H blood-group activity [19]. On the other hand, glycoproteins of hog gastric mucosa often display both (A+H) blood-group activities [1–4]. The carbohydrate chains of these glycoproteins are highly branched and contain A and H determinants linked to the common saccharide core. A typical example of such complex structures are the carbohydrate chains found [3,4] in hog gastric mucosa blood-group (A+H) sulfated glycoproteins, i.e., Gal*N*Acα1→3Gal(2←1αFuc)β1→3/4Glc*N*Acβ1→4Gal(Fucα1→2Galβ1→3/4Glc*N*Acβ1→4Gal)β1→3/6Galβ1→4Glc*N*Acβ1→4Glc*N*Ac(6←SO_3)β1→3Galβ1→3Gal*N*Ac-Ser(Thr). The fucolipids described here offer the first proof of the existence of comparable saccharide chains on the glycosphingolipids.

The isolated fucolipids not only displayed blood-group (A+H) activity of a magnitude comparable to that of (A+H) active glycoproteins, but also contained a similar number of sugar residues. Although the structures of these fucolipids remain to be determined, certain features of the saccharide chains can be suggested on the basis of immunological assays. It is certain that each of the isolated fucolipids contains A and H antigenic determinants. Since each of these determinants involves one fucose residue, and there are 2 mol fucose/mol each glycolipid, therefore each glycolipid contains one A and one H antigenic determinant. Whether the core portion of the saccharide chains of the fucolipids is linear or contains additional branches consisting of one or more sugar residues, remains to be established. In this connection, it is worth noting that complex fucolipids of the red-cell membrane [20] and hog stomach [10] are highly branched.

Acknowledgements

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