# PARTIAL CHARACTERIZATION OF A NOVEL SULFATED GLYCEROGLUCOLIPID OF THE HUMAN GASTRIC CONTENT

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## 1. Introduction

Glycolipids of the mammalian species are divided into two groups, glycosphingolipids and glycoglycerolipids, depending on the nature of the lipid core of these substances. The majority of glycolipids found in the gastric mucosa contain sphingosine, and thus belong to the glycosphingolipids. Glycoglycerolipids originally found only in the brain [1,2] more recently were also detected in testis and spermatozoa [3,4]. Despite the extensive studies of glycolipids of the gastric mucosa [5–9], little or no attention has been paid to the presence of glycolipids in the gastric content. We have isolated the glycolipids present in human gastric secretion and have studied their major component. This glycolipid is tentatively designated as a monoalkyl-monoacyl-glyceryl triglucoside sulfate.

## 2. Experimental

### 2.1. *Materials*

Pentagastrin-stimulated human gastric secretion was obtained from several healthy individuals by gastric intubation. Monogalactosyl diglyceride, glyceryl ethers, $BF_3$ and $BCl_3$ reagents were purchased from Suppelco, Inc. (Bellefonte, Pa.). Digalactosyl diglyceride was supplied by Analabs, Inc. (Nort Haeven, Ca). Silicic acid (200–400 mesh) was from Bio-Rad Laboratories (Richmond, Ca.) and silica-gel HR plates, 250 nm coating thickness, from Analtech, Inc. (Newark, De.). Standard fatty acid methyl esters, sphingosine and sphinganine were purchased from Applied Science Laboratory (State College, Pa.). Alkyl-1-chlorides were obtained from the authentic glyceryl ethers by $BCl_3$ treatment [10].

### 2.2. *Isolation of glycolipids*

Human gastric secretion (100 ml) was dialyzed extensively against distilled water and lyophilized. The lyophilizate was extracted twice, each time for 24 h with 500 ml chloroform/methanol (2:1 v/v), and filtered through a sintered glass funnel of fine porosity. The lipids contained in the combined filtrates were concentrated, dissolved in a small volume of chloroform and applied to silicic acid column (2.5 × 45 cm) equilibrated with chloroform. The column was developed first with 800 ml chloroform, followed by 1500 ml acetone, 1500 ml acetone/methanol (9:1 v/v) and finally with 2000 ml methanol. Each fraction was analyzed by thin-layer chromatography for glycolipids. The major glycolipid of the secretion, eluted from the silicic acid column with acetone, was purified to homogeneity by preparative thin-layer chromatography in chloroform/methanol/water (65:25:4 v/v/v), chloroform/methanol/water/$NH_4OH$ (65:25:3:1 v/v/v/v) and chloroform/methanol/water (65:30:8 v/v/v).

### 2.3. *Degradative procedures*

Desulfation of the isolated glycolipid was performed with 0.05 M HCl in dry methanol or with 0.05 M HCl in anhydrous tetrahydrofuran [11]. The native and desulfated glycolipid were each subjected to alkaline methanolysis with 0.3 M NaOH in chloroform/methanol (1:1 v/v) at room temperature for 1 h. After neutralization, the methyl esters of fatty acids were extracted with hexane and treated with

$BF_3$ to assure complete esterification. The methanolic phase was chromatographed on thin-layer plates developed in chloroform/methanol/water (65:35:8 v/v/v) and the recovered glycolipids were subjected to acid methanolysis. Following extraction of glyceryl ethers with hexane, the hydrolysates were analyzed for methyl glycosides. An aliquot of acid methanolysate prior to hexane extraction was dried and treated with $BCl_3$ [10]. Following extraction of alkyl chlorides with hexane, the methanolic phase was analyzed for glycerol.

Periodate oxidation of the glyceryl ether fraction derived from the above glycolipid and of glyceryl-monoalkyl standards was performed with 0.2 M sodium metaperiodate in aqueous chloroform/methanol [11].

### 2.4. *Analytical methods*

Thin-layer chromatography was performed on silica-gel HR plates activated at 120°C for 1 h. Compounds were visualized by the following reagents: orcinol, rhodizonate, ninhydrin, ammonium bisulfate and iodine vapors [11].

Methyl esters of fatty acids, glyceryl ethers and methyl glycosides were obtained by methanolysis of the glycolipid in 1.2 M methanolic HCl at 80°C for 24 h. Following extraction of glyceryl ethers and fatty acid methyl esters with hexane, the methanol phase was neutralized with silver carbonate, dried and analyzed for methyl glycosides. The glyceryl ethers contained in the hexane extract were separated from the methyl esters of fatty acids by thin-layer chromatography [3].

Gas–liquid chromatography was performed with a Beckman GC-65 instrument equipped with glass columns (8 ft × 1/8 in) packed with 3% SE-30 on chromosorb, W, AW, DMCS (80–100 mesh). For the analysis of trimethylsilyl derivatives of glycerol and methyl glycosides, the temperature was programmed at 2°C/min from 100–210°C; program from 190–270°C at 2°C/min was used for trimethylsilyl derivatives of glyceryl ethers. For the analysis of fatty acid methyl esters, alkyl chlorides and alkoxyacetaldehydes, temperature programmings were 170–270°C at 2°C/min, 130–240°C at 3°C/min and 150–260°C at 2°C/min, respectively.

Purified glycolipid was also examined for sulfatide [13], sulfate [14], alkenyl ether group [15], phosphorus [12] and sphingosine [11].

## 3. Results

Examination of a lipid extract from human gastric contents by thin-layer chromatography revealed the presence of several glycolipid components. The major glycolipid, as judged by the intensity of staining with orcinol reagent, was eluted from the silicic acid column by acetone and purified to homogeneity on thin-layer plates. The purified glycolipid (fig.1) was obtained in a yield of 42 mg/100 ml gastric content.

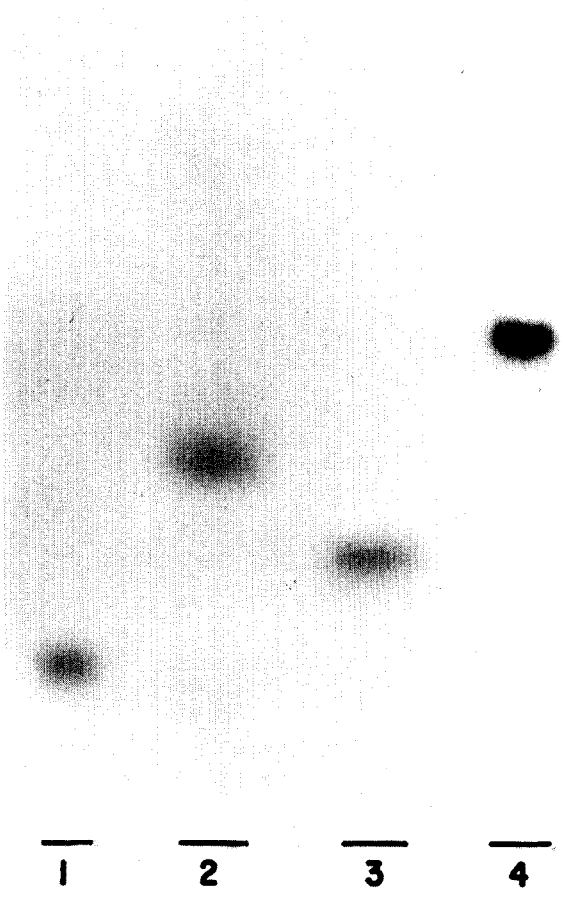


Fig.1. Thin-layer chromatogram of the major sulfated glycolipid purified from human gastric content. (1) Native glycolipid of the human gastric content. (2) Desulfated glycolipid. (3) Desulfated and deacylated glycolipid. (4) Digalactosyl diglyceride standard. Conditions: Silica-gel HR 250 nm developed in chloroform/methanol/water (65:25:4 v/v/v). Visualization: orcinol reagent.

Migration of the isolated glycolipid on thin-layer plates (fig.1) was enhanced by acid solvolysis (desulfation), whereas alkaline methanolysis (deacylation) of the native and desulfated glycolipids resulted in their conversion into more polar compounds.

Analyses of the methanolysis products of native and desulfated glycolipid revealed the presence of glucose, alkyl ethers and fatty acids. Sphingosine, phosphorus and alkenyl ethers were not detected. The native glycolipid, but not its desulfation product, reacted as a sulfatide in the assay procedure of Kean [13] and contained 1 mol sulfate/3.1 mol glucose. Gas–liquid chromatography analyses of trimethylsilyl derivatives of glycerol and methyl glucoside derived from the glycolipid revealed that these components are present in a molar ratio 1.0 : 3.07 in native glycolipid and 1.0 : 2.9 in desulfated glycolipid. These results indicate that the isolated glycolipid is a triglucosyl diglyceride sulfate.

The fatty acid composition of the studied glycolipid is given in table 1. Hexadecanoate was the major fatty acid found in the hexane extracts of acid and alkaline methanolysates. The hexane extract of acid methanolysates also contained glyceryl mono-ethers. The major glyceryl ethers, in order of abundance, were hexadecyl, octadecyl and eicosyl (table 1). Treatment of the isolated glyceryl ethers with $BCl_3$ gave alkyl chlorides, which were identified as hexadecyl-1-chloride, octadecyl-1-chloride and eicosanyl-1-chloride (table 1).

Oxidation of the glyceryl ether fraction with periodate resulted in conversion of glyceryl-mono-octadecyl ether into octadecyloxyacetaldehyde, glyceryl-monoeicosyl ether into eicosyloxyacetaldehyde, whereas glyceryl-monohexadecyl ether was not oxidized. These data indicated that the diglyceride portion of the studied glycolipid consists of a mixture of glyceryl-1-octadecyl, glyceryl-1-eicosyl and glyceryl-2-hexadecyl ethers.

Table 1
Fatty acid, glyceryl ether and alkyl group composition of the isolated glycolipid

| Fatty acid Formula | (%) | Glyceryl ether (%) | Alkyl group (%) |
|---|---|---|---|
| 14:0 | 4.2 | | |
| 16:0 | 33.1 | 37.2 | 36.8 |
| 16:1 | 2.5 | | |
| 18:0 | 12.6 | 20.1 | 19.3 |
| 18:1 | 13.1 | | |
| 20:0 | 15.4 | 26.8 | 29.7 |
| 22:0 | 3.2 | | |
| 22:1 | 3.0 | | |
| 24:0 | 2.0 | | |
| 24:1 | 3.5 | | |
| Unidentified | 7.4 | 15.9 | 14.2 |

## 4. Discussion

The major glycolipid of human gastric content has been found to contain glucose, glyceryl ethers, fatty acids and sulfate. Chemical analyses of the isolated glycolipid revealed that glucose, glycerol and sulfate are present in a molar ratio of 3:1:1 and that the glycerol ethers are of the monoalkyl type. The purified glycolipid was susceptible to deacylation, thus indicating the presence of ester-linked fatty acids. Furthermore, only glucose and glyceryl mono-ethers were detected among the products of acid methanolysis of the deacylated glycolipid. These data clearly indicated that the diglyceride portion of the glycolipid is of a monoalkyl-monoacyl type.

Glyceryl-monohexadecyl, glyceryl-mono-octadecyl and glyceryl-mono-eicosyl were found to be the principal ether components of the glycolipid. The length of the alkyl chains was also confirmed by the results of analyses of alkyl chlorides.

Results of periodate oxidation of the glyceryl mono-ether fraction indicated that the diglyceride portion of the glycolipid consists of a mixture of 1-O-alkyl and 2-O-alkyl ethers, with the former type being predominant.

Based on the data presented, we suggest that the isolated glycolipid is a monoalkyl-monoacyl-glyceryl triglucoside sulfate.

Sulfated glycolipids found thus far in mammals comprise mono-galactosyl diglyceride sulfate, galactosyl sulfatide, lactosyl sulfatide and galactosyl-lactosyl sulfatide [4,11,16]. To our knowledge, sulfate derivatives of glyceroglucolipids have not been previously described in mammalian secretions. Compounds of similar chemical composition to those described here, appear to be present in the secretions from the dog Heidenhain fundic pouch and rat stomach (Slomiany and Slomiany, unpublished observations).

It is therefore conceivable that glyceroglucolipids, because of their relatively high concentration, as compared to other glycolipids present in gastric content, form an essential component of digestive secretions of mammals.

The cellular derivation of the sulfated glyceroglucolipids has not yet been explored. The sulfated glycoproteins present in human gastric secretion are thought to arise from salivary glands [14,17], pharynx, trachea and esophagus [18] and therefore sulfated glycolipid described here may also be derived from these sources.

The sulfated glyceroglucolipids found here in the stomach of man may be involved in the defense mechanism against the injury of the mucosal surfaces by the proteolytic action of gastric proteases [19]. They may be also involved in the activation of pepsinogen into pepsin in a manner similar to that reported for other polyanions such as chondroitin sulfate or sulfated glycoprotein [20,21]. Furthermore, sulfated glyceroglucolipids, if present in the epithelial structures of the gastric mucosa, may act similarly to sulfatides, as carriers in the insorption and exsorption of sodium and potassium [22]. The active-transport of these cations through the gastric mucosa is closely related to the formation, secretion and back diffusion of $H^+$-ions, and is one of the most essential activities of the stomach.

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