PARTIAL CHARACTERIZATION OF GLYCEROGLUCOLIPIDS FROM HUMAN SALIVA

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SUMMARY - Glycolipids of human saliva have been isolated and partially characterized. The neutral glycolipids consisted of six compounds, composed of glucose, glyceryl ethers and fatty acids, and differed from each other primarily with respect to the number of glucose residues. The major acidic glycolipid contained glucose, glyceryl ethers, fatty acids and sulfate. Based on the data of chemical analyses we propose that the acidic glycolipid is a 1-O-alkyl-2-O-acylglycerol triglucoside sulfate and that the neutral glycolipids are mono-, di-, tri-, hexa- and octaglucoside derivatives of 1-O-alkyl-2-O-acylglycerol.

INTRODUCTION - The glycolipids of salivary glands and the mucosa of the gastrointestinal tract consist mainly of glycosphingolipids (1-4). The carbohydrate portion of these glycolipids exhibit a considerable degree of variance with respect to sugar composition, length of oligosaccharide chains and degree of branching (5-7). Until recently, it was assumed that glycolipids of the alimentary tract secretions are similar to, or possibly derived from, those found on the cell surfaces (8). Our recent investigations on glycolipids of the human gastric secretion showed that these substances are composed of monoalkylmonoacylglycerol and variable number of glucose residues, some of which are sulfated (9-12). Since glycoproteins of saliva and gastric secretion bear considerable structural and immunological similarities (13,14), it was interesting to learn if the glycolipids of the saliva resemble those of the gastric secretion. In this paper we describe the isolation and partial characterization of the glycolipids present in the human saliva.

MATERIALS AND METHODS - Saliva (100 ml) collected from several individuals was dialyzed against distilled water and lyophilized. Dry residue was extracted twice, each time for 24 h. with chlorofomr/methanol (2/1, v/v) and filtered through a fine porosity sintered glass funnel. The lipids were concentrated, dissolved in a small volume of chloroform and applied to silicic acid column (1.2 x 30 cm). The column was developed first with chloroform (500 ml), followed by acetone (700 ml), 700 ml of acetone/methanol (9/1,v/v) and finally with methanol (900 ml). Each fraction was monitored for glyco-

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lipids by thin-layer chromatography (9). The glycolipids, contained mainly in the acetone and acetone/methanol fractions, were further separated into individual components by thin-layer chromatography in chloroform/methanol/water (65/30/8, v/v), chloroform/acetone/methanol/water (50/40/20/5, v/v) and chloroform/methanol/acetic acid/water (60:20:20:1, v/v).

Methyl esters of fatty acids, glyceryl ethers and methyl glycosides were obtained by methanolysis of glycolipids (10). The fatty acid methyl esters were separated from glyceryl ethers by thin-layer chromatography (15). For analysis of glycerol, an aliquots of acid methanolysates prior to hexane extraction were dried and treated with BCl_3 (16). Following extraction of fatty acid methyl esters and alkyl chlorides with cold hexane, the methanolic phases were analyzed for glycerol (17). Alkyl chlorides and glycerol were also obtained from the purified glyceryl ether fractions using the above procedure. Purified glycolipids were also examined for sulfatide (18), sulfate (19), alkenyl ether group (20), phosphorus (21) and sphingosine (22). Compounds were visualized on thin-layer plates by the following reagents: orcinol, rhodizonate, ninhydrin, ammonium bisulfate and iodine vapors (9).

Desulfation was performed with 0.05 M HCl in dry methanol or with 0.05 M HCl in anhydrous tetrahydrofuran (9). Mild alkaline methanolysis was performed 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. Glycolipids recovered from the reaction mixtures were chromatographed on thin-layer plates (12) and subjected to acid methanolysis. Following extraction of glyceryl ethers with hexane, the methanolic phases were analyzed for methyl glycosides (17).

Periodate oxidation of glyceryl ethers derived from the purified glycolipids was performed with 0.2 M sodium metaperiodate in aqueous chloroform/methanol at room temperature for 24 h. (12). Alkoxyacetaldehyde references were obtained from glyceryl-1-O-alkyl standards by the above procedure. Alkyl-1-chlorides were obtained from the authentic glyceryl ethers by BCl_3 treatment (16).

Gas-liquid chromatography analyses of trimethylsilyl derivatives of glycerol and methyl glycosides were performed on the columns (180 x 0.2 cm) packed with 3% SE-30 on chromosorb, W, AW, DMCS (80-100 mesh) 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, with or without silylation of the 2-hydroxy group, 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. The alkyl chlorides, glyceryl ethers and alkoxyacetaldehydes of a higher carbon number than C_{18} were identified from the semilogarithmic plot of carbon number versus log of the retention time.

RESULTS - Thin-layer chromatography of the lipid fractions from silic acid column revealed the presence of eight glycolipid components in the acetone eluate, whereas the acetone/methanol fraction gave one major and two minor glycolipid bands. After rigorous purification of the glycolipids present in both fractions, seven individual components (Fig. 1) were isolated. Glyco-

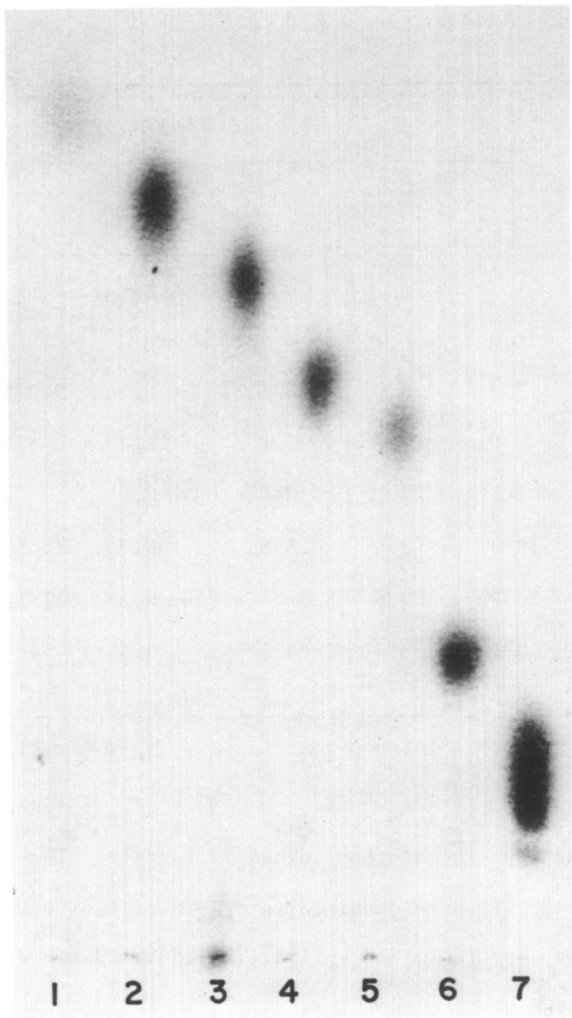

Figure 1. Thin-layer chromatography of the glycolipids purified from human saliva.

1, Glycolipid I; 2, glycolipid II; 3, glycolipid III; 4, glycolipid IV; 5, desulfated glycolipid V; 6, glycolipid VI; 7, glycolipid VII (major band) and its deacylation product (minor band). Conditions: Silica gel HR 250 nm developed in chloroform/methanol/water (65/30/8, v/v). Visualization: orcinol spray.

lipids I - V were present exclusively in the acetone eluate, glycolipid VI was found in both acetone and acetone/methanol eluates, whereas glycolipid VII

Table I. Glyceryl ether composition of the isolated glycolipids.

Short hand formula	Glycolipid						
	I	II	III	IV	V	VI	VII
				% Total			
14:0	5.0	6.4	9.3	10.6	-	26.1	16.4
16:0	7.2	5.9	26.3	8.9	9.2	21.1	18.2
18:0	2.0	4.4	5.1	1.4	-	-	0.3
20:0	2.5	27.1	12.6	2.2	-	-	7.1
21:0	18.0	22.9	18.8	60.7	73.5	9.3	10.9
22:0	56.2	25.3	21.6	9.7	12.1	34.5	39.1
unidentified	9.1	8.0	4.3	4.5	5.2	9.0	8.0

was the major component of the acetone/methanol fraction. The purified glycolipids I, IV, V, VI and VII were obtained in a yield of 0.6, 0.9, 1.2, 2.8 and 4.7 mg., respectively, per 100 ml of saliva. The combined yield of glycolipids II and III was 1.56 mg.

Analyses of the methanolysis products of the isolated glycolipids revealed the presence of glucose, glyceryl ethers and fatty acids. One of the glycolipids, glycolipid V, reacted as sulfatide in the assay procedure of Kean (18) and contained 0.9 mole of sulfate per one mole of glycerol. None of the studied glycolipids contained sphingosine, phosphorus and alkenyl ethers.

Gas-liquid chromatography of trimethylsilyl derivatives of glycerol and methyl glucoside showed that these components are present in a molar ratio of 1:1.1 in glycolipid I, 1:2.1 in glycolipids II and III, 1:2.9 in glycolipid IV and V, 1:5.9 in glycolipid VI and 1:7.8 in glycolipid VII. Although the

glycerol and glucose components of glycolipids II and III were present in identical molar ratios, these compounds differed in the composition of their diglyceride portions. The major glyceryl mono-ethers of glycolipid II were eicosyl, heneicosyl and docosyl, whereas hexadecyl and docosyl were predominant in glycolipid III. The glyceryl ether composition of the isolated glycolipids is given in Table I.

Periodate oxidation of the glyceryl ether fractions, obtained from the individual glycolipids, resulted in their conversion (over 85%) to corresponding alkoxyacetaldehydes. Treatment of the glyceryl ether fractions with BCl_3 gave alkyl chlorides which corresponded in composition to glyceryl mono-ethers of the individual glycolipids. These data indicate that the diglyceride portion of the studied glycolipids is mainly composed of 1-O-alkyl-2-O-acylglycerol.

The acyl components of diglyceride portion of the isolated glycolipids consisted mainly of eicosanoate, docosanoate and tetracosanoate, which together with their 2-hydroxy derivatives constituted over 70% of the total fatty acids. Methanolysates of the deacylated glycolipids were devoid of fatty acids.

DISCUSSION - Seven individual glyclipids (I - VII) have been isolated from the lipid extract of the saliva and their composition were determined. These compounds were found to contain diglyceride lipid core and variable number of glucose residues, some of which (glycolipid V) contained sulfate. The 1-O-alkyl-2-O-acylglycerol nature of the diglyceride core was established from the results of analysis of glyceryl ethers and fatty acids, before and after deacylation. The point of attachment of the alkyl and acyl groups to the glycerol was elucidated from the periodate oxidation data, i.e., conversion of the glyceryl mono-ethers to the corresponding alkoxyacetaldehydes.

Based on the molar ratios of glycerol to glucose, we suggest that these glycolipids are monoglucosyl diglyceride (glycolipid I), diglucosyl diglyceride (glycolipids II and III), triglucosyl diglyceride (glycolipid IV), triglucosyl diglyceride monosulfate (glycolipid V), hexaglucosyl diglyceride (glycolipid VI) and octaglucosyl diglyceride (glycolipid VII). Thus, the glyco-

lipids of the saliva show considerable chemical similarities to glycerogluco-lipids of the gastric secretion, structures of which were elucidated recently (11,12).

The data presented here together with our previous studies on glycolipids of gastric secretion (9-12) strongly indicate that glycolipids of the mucous secretions are entirely different from those found on the cell membrane. It is therefore conceivable that the mucous secretions of the pulmonary and reproductive tracts also contain glyceroglucolipids.

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