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Plasmalogen Composition of *Anaeroplasma*

T. A. LANGWORTHY,* W. R. MAYBERRY, P. F. SMITH, AND I. M. ROBINSON[1]
Department of Microbiology, University of South Dakota, Vermillion, South Dakota 57069

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The polar lipids of *Anaeroplasma* contained 33.1% alk-1'-enyl glyceryl ether (plasmalogen) form. Phosphatidylglycerol was the major polar lipid (55.2%) and contained nearly all of the plasmalogen. The alk-1'-enyl glyceryl ether form accounted for 58.3% of the phosphatidylglycerol.

The presence of plasmalogens (alk-1'-enyl glyceryl ethers) in the lipids of several species of anaerobic bacteria has been firmly established (3). The occurrence of plasmalogens among members of the *Mycoplasmatales*, however, has not been described. We present here evidence for the existence of plasmalogens among the lipids of two strains of obligately anaerobic mycoplasmas designated *Anaeroplasma* (I. M. Robinson, and M. J. Robinson, M. J. Allison, and P. A. Hartman, submitted for publication).

An obligately anaerobic rumen mycoplasma possessing lytic enzymes that cause digestion of gram-negative bacteria has been isolated and characterized by Robinson and Hungate (8). Using these methods, a similar bacteriolytic mycoplasma, *Anaeroplasma bactoclasticum* (Robinson and Allison, submitted for publication), was isolated from the rumens of cattle and sheep and designated strain 7LA. In addition, an anaerobic mycoplasma, *Anaeroplasma abactoclasticum* (Robinson, Allison, and Hartman, submitted for publication), not capable of digesting bacterial cells, was isolated and designated strain 6-1 (deposited in the American Type Culture Collection as ATCC 27879). Both organisms were examined for the presence of plasmalogens.

The organisms were grown anaerobically at 37 C for 18 h in the mineral salts, soluble-starch, rumen fluid medium described by Caldwell and Bryant (1). The medium was modified by increasing the concentrations of glucose, cellulose, and starch to 0.2%, and deleting agar and Na_2S. Cells were harvested, washed, and lyophilized, and lipids were extracted with chloroform-methanol (2:1 [vol/vol]). The total lipids were separated into major classes by fractionation on silicic acid columns (7, 10). Total extractable lipids accounted for about 15.5% of the cell (dry weight). The distribution between neutral lipids, glycolipids, and polar lipids from each organism is shown in Table 1. The bacteriolytic strain 7LA contained only about 50% of the amount of glycolipids present in the non-bacteriolytic strain 6-1.

Each of the separated lipid classes was subjected to acid methanolysis, followed by saponification to release fatty acids and aldehydogenic chains as the dimethyl acetals (4). Free aldehydes were obtained by mild acid hydrolysis in either 90% acetic acid or 0.1 N HCl at 100 C for 45 min. (4). The aldehyde and fatty acid compositions of each lipid class were determined by gas-liquid chromatography on columns of 5.5% SE-30 on 80- to 100-mesh Gas Chrom Q, 5.5% Dexsil 300 on Gas Chrom Q and 10% polyethylene glycol 1000 on Chromosorb W (7). Aldehydogenic chains were determined as the free aldehydes, the dimethyl acetal derivatives, and as the alcohol derivatives after reduction of the free aldehydes with sodium borohydride. Fatty acids were determined as the methyl esters.

Fatty acid methyl esters were tentatively identified from a plot of log relative retention time versus chain length generated using the NIH-D standard for the normal saturated and

TABLE. 1. *Lipid composition of Anaeroplasma*[a]

Lipid class	Dry wt of organism (%)		Total lipid (%)	
	6-1	7LA	6-1	7LA
Total lipids	15.36	16.26	100	100
Neutral lipids	3.3	6.3	21.5	38.8
Glycolipids	6.6	3.6	43.5	22.9
Polar lipids	5.4	6.3	34.9	38.9

[a] Strain 6-1, *A. abactoclasticum;* strain 7LA, *A. bactoclasticum*.

[1] Present address: National Animal Disease Center, Agricultural Research Service, Ames, Iowa 50010.

unsaturated esters. The methyl esters of the total lipid of *Staphylococcus aureus* were used for the branched chain esters (13). The fatty aldehydes were tentatively identified in a similar fashion, and the alcohols, in comparison to plots generated using alcohols prepared from the NIH-D and *S. aureus* methyl esters by sodium reduction.

The fatty acid and aldehyde distribution in the major lipid classes of the non-bacteriolytic strain 6-1 is shown in Table 2. Aldehydes were detected only in the polar lipids. The major aldehydes and fatty acids found were the saturated 16 and 18 carbon compounds. The bacteriolytic strain 7LA had a similar aldehyde and fatty acid composition.

The polar lipids from each strain of mycoplasma were identified using the methods described previously (7, 10). Phospholipids were separated by thin-layer chromatography on Silica Gel H developed in chloroform/methanol/water (65:25:4 [vol/vol/vol]). The phospholipids were detected with the reagent of Vaskovsky and Kostetsky (12) and by radioautography after cells had been grown in the presence of ^{32}P inorganic orthophosphate. Both organisms had the same phospholipid distribution. A major phospholipid was present which accounted for 55.2% of the lipid phosphorus. It chromatographed as phosphatidylglycerol and gave a positive reaction with 2,4-dinitrophenylhydrazine reagent (9) or Schiff reagent after exposure to HCl fumes (6). Other phospholipids included a ninhydrin-positive component which migrated as an amino acyl phosphatidylglycerol (27.3%) and several minor phospholipids, one of which migrated as cardiolipin (4.9%).

TABLE 2. *Fattly acid ester and aldehyde composition of lipids from Anaeroplasma abactoclasticum*

Chain Length[a]	Fatty acids[b] (mol %)			Aldehydes[c] (mol %)
	Neutral Lipids	Glyco-lipids	Polar Lipids	Polar Lipids
12:0	0.9	1.6	0.9	
13:br		0.8	1.3	
14:br		1.5	1.6	
14:0	3.3	4.1	2.6	1.3
15:br	2.9	5.9	10.6	0.9
15:0	3.0	4.4	3.4	3.4
16:0	20.6	30.6	14.5	24.3
17:br		0.9	0.7	1.9
17:0	1.6	1.2	1.3	7.3
18Δ	13.6	5.3	12.0	
18:0	43.5	35.7	34.3	59.3

[a] First number represents length of carbon chain; 0, saturated; Δ, double bond; br, branched.

[b] 19:0, 20Δ, and 20:0 fatty acids were present in trace amounts.

[c] Aldehydes were detected only in the polar lipids. Aldogenic chains were determined as the free aldehydes; as the dimethyl acetal derivatives and as the alcohol derivatives.

After preparative thin-layer chromatography, each phospholipid was subjected to mild alkaline hydrolysis (2). The water-soluble products were chromatographed (ascending) together with similarly prepared standards on Whatman no. 1 filter paper developed in *n*-propanol/ammonia/water (6:3:1 [vol/vol/vol]) and isopropanol/ammonia/water 7:2:1 [vol/vol/vol]). The glycerolphosphoryl esters were detected both by radioautography and with the reagent of Hanes and Isherwood (5).

The amount of alk-1'-enyl glyceryl ethers in each phospholipid was estimated by applying individual phospholipids to thin-layer chromotography plates, spraying with mercuric chloride (9) or exposing to HCl fumes (6), and finally developing in chloroform/methanol/water (65:25:4 [vol/vol/vol]). The phosphate was estimated in the diacyl and any monoacyl phospholipid formed. Comparable results were obtained using the method described by Gray (4). Alk-1'-enyl glyceryl ethers were detected only in phosphatidylglycerol. The alk-1'-enyl glyceryl ether form accounted for 58.3% of the phosphatidylglycerol and 33.1% of the total lipid phosphorus. The fatty acid and aldehyde composition of the phosphatidylglycerol was identical to the total polar lipids shown in Table 2.

Both of the obligately anaerobic organisms, *A. bactoclasticum* and *A. abactoclasticum* contain plasmalogen, but otherwise have a lipid composition similar to the aerobic members of the *Mycoplasmatales* (11).

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