Lipids of Sphaerophorus ridiculosis: Plasmalogen Composition

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Lipid analyses of the anaerobic bacterium Sphaerophorus ridiculosis revealed that 24.2% of the polar lipids are the alk-1'-enyl glyceryl ether (plasmalogen) form. The major polar lipids, phosphatidylethanolamine (67.5%), phosphatidylglycerol (11.2%), cardiolipin (12.0%), and lyso-phosphatidylethanolamine (9.3%), contained 26.3, 7.8, 5.2, and 13.4% plasmalogen, respectively.

Glyceryl ethers are present in most mammalian tissues (17, 21) but have until recently been isolated from only a few anaerobic microorganisms (1, 4, 8, 9). Recently, Kamio et al. (12) have shown that these lipids are present in a number of anaerobic soil microorganisms, and Meyer and Meyer (14) have reported alk-1'-enyl glyceryl ethers (plasmalagens) in Treponema pallidum (Reiter) isolated from soil. It seems evident, therefore, that ether-containing lipids are widespread among the anaerobic bacteria.

We report here the identification of alk-1'-enyl glyceryl ethers and the possible presence of alkyl glyceryl ethers in a species of the gram-negative, anaerobic, highly pleomorphic bacteria Sphaerophorus most closely resembling Sphaerophorus ridiculosis.

The organism (isolated 13 June 1968) was isolated and grown to late-exponential growth phase as previously described (15). The cells were harvested and washed, and the lipids were extracted as described (7). About 4.2% of the dry weight of the cells was lipid extractable, as determined by the Folch et al. (6) extraction procedure.

The total lipids were separated by silicic acid column chromatography into neutral lipids (16.2%), glycolipids (1.4%), and polar lipids (82.4%) (10). The glycolipids were not investigated further. The neutral lipids were separated by thin-layer chromatography (TLC) on Silica Gel G in hexane-diethylether-acetic acid (80:20:1 or 50:50:2, vol/vol/vol) or hexane-chloroform-ethanol (135:60:5, vol/vol/vol) and were identified by co-chromatography with the appropriate standards (Superco, Analabs, Nu-Chek Prep). The major neutral lipids, free fatty acids (61.4%), triglycerides (11%), and diglycerides (2%), were quantitated by the method of Privett et al. (16). A compound (9%) which co-chromatographed with cholesterol and showed pink coloration after H2SO4 spray has not been further identified. Trace amounts of monoglycerides, wax esters, and long-chain fatty alcohols were also present.

The total polar lipids were subjected to LiAlH4 reduction (24), and the reaction products were determined by TLC (24). Photodensiometric determinations (16) indicated approximately 18% of the total polar lipid to be the alk-1'-enyl glyceryl ether form. A lipid which yields a spot when sprayed with H2SO4 spray (16) co-chromatographed with alkyl glyceryl ethers and represented 1.4% of the total lipids. Whether this band is composed of 1, 2- or 1, 3-alkane diols or both in addition to alkyl glyceryl ethers, as observed in Clostridium butyricum (9) lipids, is at present unknown.

The alk-1'-enyl glycerols obtained were hydrogenated in the presence of Adams catalyst, and the resulting alkyl glycerol ethers were shown to co-chromatograph with 1-O-alkyl glycerol ether (gift from Randell Wood) on sodium arsenite-impregnated, silica gel thin-layer plates (23). This suggests the structure of 1-O-alkyl-2-acyl-3 glyceryl phosphatidate for these lipids.

Table 1 shows the carbon number of the aldehydes liberated by acid from the 1-O-alk-1'-enyl glycerols (2) and the acetate derivatives (19) of the fatty acids obtained by LiAlH4 reduction of the polar lipids. The aldehydogenic chain is relatively simple and predominantly saturated.

The major polar lipids were shown by TLC on silica gel HRB (Brinkman), developed in chloroform-methanol-acetic acid-0.9% sodium chloride (85:25:8:4, vol/vol/vol/vol) or chloroform-methanol-ammonium hydroxide (65:35:5, vol/vol/vol), to co-chromatograph with phosphatidylethanolamine, lyso-phosphatidylethanolamine, phosphatidylglycerol, and cardi-
olipin. After preparative TLC separations, the bands were visualized under ultraviolet light after spraying with 1,2-dichlorofluorescein, and the lipids were eluted from the silicic acid as described (4). The percent phosphorus (3) of the separated polar lipid is shown in Table 2. Identical percentages were obtained when the cells were grown to late-exponential growth phase in the presence of sup35 inorganic orthophosphate, and the radioactivity in the separated polar lipids was determined by zonal scanning (18) of the developed TLC plate. Identification of the polar lipids was confirmed chromatographically with sup35-labeled polar lipids.

Each phosphatide was subjected to sequential mild alkaline (4) and acid (5) hydrolysis. The water-soluble products were chromatographed (ascending) against similarly prepared standards on Whatman no. 1 filter paper in n-butyl alcohol-acetic acid-ethyl alcohol (5:4:1, vol/vol/vol) and phenol-water-acetic acid-ethyl alcohol (50:22:3:3, vol/vol/vol) as described (7). The glycerylphosphoryl esters were identified either by radioautography, by scintillation counting of 1-cm strips of the chromatogram, or by the reagent of Hanes and Isherwood (11) or the modified Wade and Morgan reagent (20). Certain of the TLC-separated polar lipids contained small amounts of either one or both of the neighboring lipids. However, we could not detect any other phosphatide by these techniques.

The amount of alk-1'-enyl glyceryl ethers of each phosphatide was determined as mild alkaline stable lipid (4) by isotope or phosphorus determination, as well as by the method of Williams et al. (22). Comparable results were obtained by these techniques (Table 3). Determinations of the total polar lipids (4, 22) showed that 24.2% were in the alk-1'-enyl glyceryl ether form.

The lipid composition of this species of Sphaerophorus is not unusual (13). The presence of an O-alk-1'-enyl lysophosphatidylethanolamine has, however, not been reported previously. We do not believe that this compound is an artifact due to the isolation procedure, since identical procedures of lipid isolation from C. butyricum give no indication of lysoplasmalogens (10).

This report supports the contention that most anaerobic bacteria contain ether lipids. At present it is puzzling that the ability to synthesize these lipids appears to have been lost in aerobic bacteria, yeast, fungi, and plants, whereas invertebrates and certain mammalian tissues synthesize substantial quantities of these lipids (8).

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LITERATURE CITED

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### Table 1. Chain length of the aldehydogenic and esterified acids of S. ridiculosis polar lipids

<table>
<thead>
<tr>
<th>Chain</th>
<th>Aldehydes (wt, %)</th>
<th>Fatty acids (wt, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0</td>
<td>Trace</td>
<td>1.2</td>
</tr>
<tr>
<td>14:0</td>
<td>12.8</td>
<td>27.1</td>
</tr>
<tr>
<td>16:0</td>
<td>71.0</td>
<td>35.6</td>
</tr>
<tr>
<td>16:1</td>
<td>16.2</td>
<td>30.4</td>
</tr>
<tr>
<td>18:0</td>
<td>Trace</td>
<td>0.9</td>
</tr>
<tr>
<td>18:1</td>
<td>Trace</td>
<td>4.8</td>
</tr>
<tr>
<td>Total saturated</td>
<td>83.8</td>
<td>64.8</td>
</tr>
<tr>
<td>Total unsaturated</td>
<td>16.2</td>
<td>35.2</td>
</tr>
</tbody>
</table>

* Polar lipids reduced with LiAlH4. The products O-alk-1'-enyl glycerol and fatty alcohols were isolated by TLC (24).

* Obtained by acid hydrolysis of the LiAlH4 reduction product O-Alk-1'-enyl glycerol.

* Acetate derivatives of the fatty alcohols were obtained by LiAlH4 reduction of polar lipids.

### Table 2. Composition of major phosphatides of S. ridiculosis

<table>
<thead>
<tr>
<th>Compound</th>
<th>Content (% ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyso-phosphatidylethanolamine</td>
<td>9.3 ± 1.5</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>67.5 ± 1.6</td>
</tr>
<tr>
<td>Phosphatidylglycerol</td>
<td>11.2 ± 1.1</td>
</tr>
<tr>
<td>Cardiolipin</td>
<td>12.0 ± 0.7</td>
</tr>
</tbody>
</table>

* Percent total polar lipids isolated from late-exponential growth phase cells.

* SD, standard deviation.

### Table 3. Proportions of S. ridiculosis phosphates in plasmalogen form

<table>
<thead>
<tr>
<th>Compound</th>
<th>Plasmalogen form (% ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyso-phosphatidylethanolamine</td>
<td>13.4 ± 5.2</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>26.3 ± 4.8</td>
</tr>
<tr>
<td>Phosphatidylglycerol</td>
<td>7.8 ± 1.9</td>
</tr>
<tr>
<td>Cardiolipin</td>
<td>5.2 ± 1.6</td>
</tr>
</tbody>
</table>

* Percent plasmalogen in individual phosphatides isolated from late-exponential growth phase cells.

* Determined by the method of Williams et al. (22). SD, standard deviation.