A Simple Chromatographic Procedure for the Detection of Cyclized Archaebacterial Glycerol-Bisdiphytanyl-Glycerol Tetraether Core Lipids

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Archaebacterial glycerol-bisdiphytanyl-glycerol tetraether core lipids containing from one to eight cyclopentane rings could be resolved from each other and from the parent uncyclized C40,C40 lipid by TLC. The core lipids of examples from the genera Methanobacterium, Methanobrevibacter, Methanogenium and Methanoplanus did not contain cyclized forms of glycerol-bisdiphytanyl-glycerol tetraethers, whereas the core lipids of Methanosarcina barkeri contained glycerol-bisdiphytanyl-glycerol tetraethers with from one to three cyclopentane rings in each C40 isopranoid chain.

INTRODUCTION

All membrane lipids of archaebacteria characterized to date are based on saturated isopranoid ethers formed by condensation of glycerol, or more complex polyols, with isopranoid alcohols containing 20, 25 or 40 carbon atoms (De Rosa et al., 1986a). Most halophilic archaebacteria have membrane lipids based exclusively on 2,3 di-O-phytanyl-sn-8lycerol (C20,C20), although a few isolates, notably the alkaliphiles, also have significant amounts of C25 (sesterterpanyl) chains as C20,C25 glycerol diethers (De Rosa et al., 1982, 1983a). The situation is more complex for methanogenic and thermophilic archaebacteria, since, in most cases, the membranes have a significant component of glycerol-bisdiphytanyl-glycerol tetraethers. These are formed by dimerization of diphtanyol diethers, where head-to-head linkage between the terminal methyls occurs.

Methanogen core lipid structures are particularly diverse, in that in some isolates, glycerol may be replaced by a tetritol, both in the core lipid structures (De Rosa et al., 1986b) and in complex lipids (Ferrante et al., 1987). Furthermore, one isolate has a macrocyclic diether with a 36-member ring, which originates from the condensation of a glycerol, in the 2,3-sn-configuration, with the 3,7,11,15,18,22,26,30-octamethyldotriacontane-1-32-diol (Comita & Gagman, 1983).

Thermophilic archaebacteria of the genera Desulfurococccus, Thermoproteus, Thermofilum and Pyrodictium have ether lipids based on C20 and C40 chains (Stetter et al., 1983; Zillig et al., 1981, 1982, 1983) whereas Thermococcus celer contains only C20,C20 lipids (De Rosa et al., 1987). In Sulfolobus spp. the lipids are based on C40,C40 tetraethers, but there are two classes; one class consists of glycerol dialkyl glycerol tetraethers (GDGT), while the second differs from the first in that, nonitol, a nine-carbon polyol, replaces one of the glycerols (GDNT). These C40,C40 lipids have the additional feature of containing up to four cyclopentane rings in each C40 chain. The degree of cyclization depends on the growth temperature of the organism (De Rosa et al., 1986a).

Abbreviations: GDGT, glycerol dialkyl glycerol tetraethers; GDNT, glycerol dialkyl nonitol tetraethers.

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Cyclization of C_{40},C_{40} core lipids has only been found in one methanogen to date (De Rosa et al., 1986b), but detailed structural analyses of C_{40},C_{40} lipids have only rarely been done (De Rosa et al., 1986b; Kramer et al., 1987; Kushwaha et al., 1981; Comita & Gagosian, 1983). The resolution and identification of cyclized forms currently involves a lengthy combination of column chromatography and HPLC, or GLC of the hydrocarbon chains after hydrolysis and derivatization (De Rosa et al., 1983b; Tornabene & Langworthy, 1979; Makula & Singer, 1978). We report here a simple TLC procedure for the resolution of different cyclized forms of GDGT and the use of this procedure to survey the core lipids of a number of different methanogens.

**METHODS**

Micro-organisms and culture conditions. Details of methanogens are shown in Table 1. Growth conditions were as previously described (Grant et al., 1985). Sulfolobus solfataricus MT-4 was grown in the standard medium at 87 °C as previously described (De Rosa et al., 1975). Cells were harvested at the stationary phase of growth and freeze-dried.

Extraction and isolation of lipids. Polar lipids were extracted by the procedure of Bligh & Dyer (1959) as modified by Minnikin et al. (1979). Core lipids were extracted into chloroform or hexane, after hydrolysis of complex lipids by acid methanolysis (Ross et al., 1985). Purified samples of 2,3-di-O-phytanyl-sn-glycerol (C_{20},C_{20} diether), 3-O-phytanyl-sn-glycerol (C_{20} monoether), tetritol C_{20},C_{20} diether, uncyclized C_{40},C_{40} tetraether and the glycerol-bisdiphytanyl-glycerol tetraether (C_{40},C_{40} tetraether) fraction from Methanosarcina barkeri (De Rosa et al., 1986b) were available for comparison by TLC.

TLC. This was done on 0.25 nm layers of silica gel F 254 (Merck), activated by heating at 100 °C for 2 h. The solvent was n-hexane/ethyl acetate (7:3, v/v), single development. Compounds were detected by spraying with 0.1% (w/v) Ce(SO₄)₂ in 1 M-H₂SO₄, followed by heating at 150 °C for 5 min.

**RESULTS AND DISCUSSION**

In a previous study (Grant et al., 1985) the core lipids of a considerable number of different methanogens were analysed by TLC. Glycerol ethers with 20, 25 and 40 carbon atoms were detected, along with a number of new structures. In a later study, two of these new structures from *Methanosarcina barkeri* were characterized as a C_{20} glycerol monoether and C_{20},C_{20} tetritol diether (De Rosa et al., 1986b). Furthermore, the C_{40},C_{40} tetraether core lipids of this organism were shown to be of the GDGT class and to contain from one to three cyclopentane rings (De Rosa et al., 1986b). To date, *Methanosarcina barkeri* is the only known methanogen known to possess cyclized core lipids; indeed this organism contains no uncyclized C_{40},C_{40} lipids. The TLC system used by

<table>
<thead>
<tr>
<th>Culture collection no.*</th>
<th>Organism</th>
<th>Core lipids</th>
<th>C_{40},C_{40}</th>
<th>C_{20},C_{20}†</th>
</tr>
</thead>
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<tr>
<td>DSM 862</td>
<td><em>Methanobacterium bryantii</em></td>
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<td>+</td>
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</tr>
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<td>+</td>
<td></td>
</tr>
<tr>
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<td>+</td>
<td></td>
</tr>
<tr>
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<td>+</td>
<td></td>
</tr>
<tr>
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<td>+</td>
<td></td>
</tr>
<tr>
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<td>—</td>
<td>+</td>
<td></td>
</tr>
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<td>—</td>
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</tr>
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<tr>
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<tr>
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</tr>
<tr>
<td>DSM 2279</td>
<td>Methanoplanus limicola</td>
<td>Acyclic</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>DSM 800</td>
<td>Methanosarcina barkeri</td>
<td>Cyclic</td>
<td>+†</td>
<td></td>
</tr>
</tbody>
</table>

—, No C_{40},C_{40} components present.

* Data from Grant et al. (1985).

† Also contains C_{20} glycerol monoether, C_{25},C_{20} glycerol diether and C_{20},C_{20} tetritol diether (De Rosa et al., 1986b).
Fig. 1. TLC of core ether lipids using a simple development system (n-hexane/ethyl acetate, 7:3, v/v). Lanes: A, 2,3-di-O-phytanyl-sn-glycerol; B, uncyclized C40,C40 derivative of GDGT; C, core lipids of *S. solfataricus* (structures of compounds 1–9 are indicated in Fig. 2); D, C40,C40 lipids of *M. barkeri*; E, 3-O-phytanyl-sn-glycerol (lower band) and tetritol C20,C20 diether (upper band).

Grant *et al.* (1985) does not resolve cyclized forms of C40,C40 core lipids from uncyclized forms and it is, therefore, not clear whether cyclized C40,C40 core lipids are present in the hydrolysed lipid extracts of those methanogens that have not been subject to detailed chemical characterization.

Chromatography of core lipid extracts on 0.25 mm layers of silica gel F 254 in n-hexane/ethyl acetate 7:3 (v/v) resolved both uncyclized GDGT and the eight different cyclized variations of this C40,C40 tetraether lipid found in the core lipids of *S. solfataricus* (Fig. 1). The structures of these compounds are shown in Fig. 2. Purified samples (De Rosa *et al.*, 1983b) were used to confirm the separation order shown in Fig. 1. A purified C40,C40 core lipid fraction from *M. barkeri* was readily resolved into the three fractions corresponding to the cyclized forms of GDGT containing four, five or six cyclopentane rings, characterized in the previous study (De Rosa *et al.*, 1986b). Samples of C20,C20 diether, tetritol C20,C20 diether and C20 monoether have different *Rf* values and were clearly distinct from C40,C40 tetraethers (Fig. 1). GDNT ethers remained at the origin using this solvent system.

Table 1 shows the results of a survey of the core lipids of several different methanogens using this TLC procedure. Of the strains tested, only *M. barkeri* possessed cyclized GDGT core lipids.
Fig. 2. Structures of C_{40},C_{40} core lipids (GDGT type), the backbone of complex lipids of thermophilic archaebacteria. The numbers 1–9 correspond to the bands in Fig. 1, lane C.

Lipids. Included in the survey were *Methanobacterium thermoautotrophicum* and *Methanobrevibacter arboriphilicus*, whose complex lipids have been extensively characterized as C_{20},C_{20} diethers and uncyclized C_{40},C_{40} (GDGT) derivatives (Kramer et al., 1987; Morii et al., 1986). This preliminary survey suggests that cyclization of C_{40},C_{40} tetraether lipids is likely to be uncommon amongst methanogenic archaebacteria that contain C_{40},C_{40} tetraether lipids of the GDGT class. This supports and extends the results of the early surveys of Tornabene & Langworthy (1979) and Makula & Singer (1978).

The pattern of complex lipids and core lipids of archaebacteria has some value in the taxonomy of the group (Ross et al., 1985; Langworthy & Pond, 1986; Grant et al., 1985). The use of this simple TLC procedure for the detection of cyclized GDGT core lipids extends the range of techniques that can readily be applied in non-specialist laboratories.

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REFERENCES


TLC of cyclized archaebacterial lipids


