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Novel Complex Polar Lipids from the Methanogenic Archaebacterium *Methanospirillum hungatei*

Abstract. *The methanogenic archaebacterium* Methanospirillum hungatei *contains two unusual phosphoglycolipids that account for 64 percent of the total cellular lipids. These lipids are derivatives of the dibiphytanyl diglycerol tetraether, previously identified in methanogens. One of the free hydroxyls of this tetraether is esterified with glycerophosphoric acid, and the other is linked glycosidically to a disaccharide. The two phosphoglycolipids may function as covalently bonded lipid bilayers to impart stability and rigidity to methanogen membranes.*

Methanogenic bacteria are strict anaerobes, most of which utilize hydrogen and carbon dioxide to produce methane (*1*, *2*). Together with certain thermoacidophilic bacteria and the extremely halophilic bacteria, they are considered to represent a line of early divergence from the eukaryotic and eubacterial lines of evolutionary descent and are designated archaebacteria (*3*). Two recent reports (*4*, *5*) have shown that the lipids of methanogens are derived from phytanyl glycerol diether, previously identified in extremely halophilic bacteria (*6*), as well as from the biphytanyl diglycerol tetraether (C_{40}-tetraether), previously identified in thermoacidophiles (*7*). We now report the structural identification of the complex polar lipids in the methanogen *Methanospirillum hungatei* for comparison with those of extreme halophiles (*6*) and thermoacidophiles (*7*).

Seven polar lipid components were detected (Table 1): two phosphoglycolipids (PGL-I and PGL-II) derived from the C_{40}-tetraether; two glycolipids (DGT-I and DGT-II) derived from the C_{40}-tetraether; two glycolipids (DGD-I and DGD-II) derived from di-*O*-phytanyl glycerol; and a phospholipid (PG), the diphytanyl glycerol ether analog of phosphatidylglycerol. Both of the C_{40}-tetraether phosphoglycolipids are asymmetric structures with sugar residues on one side and the phosphoglycerol group on the other side of the tetraether moiety (Fig. 1).

Cells of *M. hungatei* were grown (*1*, *8*) and extracted by a modification (*9*) of the method of Bligh and Dyer (*10*). Total lipids (5.5 percent of cell, dry weight) were fractionated on a silicic acid column (Bio-sil A, 100–200 mesh) eluted with chloroform to remove neutral lipids, acetone to remove glycolipids, and a mixture of chloroform and methanol (3:2, by volume) to remove phosphoglycolipids and phospholipids. The individual polar lipid components were isolated in pure form by preparative thin-layer chromatography of the above fractions (see Table 1).

Lipids were hydrolyzed by first reacting them with anhydrous 2.5 percent methanolic HCl and then with 1*N* aqueous HCl; the water-soluble products (sugars and glycerophosphates) were identified by paper chromatography in a mixture of pyridine, ethyl acetate, and water (2:5:5, by volume, upper phase) (*9*). The lipid products (phytanyl diether and biphytanyl tetraether) were identified by thin-layer chromatography on silica gel G in a mixture of petroleum ether, ethyl ether, and acetic acid (50:50:1, by volume), by infrared and nuclear magnetic resonance (NMR) spectrometry, and by optical rotation and comparison with authentic standards (*6*, *7*). After hydrolysis of phosphoglycolipids in 1*N* methanolic NaOH, water-soluble products were analyzed for α-, β-, and *sn*-3 glycerophosphates (*11*, *12*), and lipid products were identified by thin-layer chromatography in a mixture of chloroform, methanol, acetic acid , and water (85:15:10:3, by volume). Phosphoglycolipids (free acid form) and glycolipids were completely methylated (*13*), and the partially methylated sugars were identified as their alditol acetates (*14*) by gas chromatography and mass spectrometry (*15*).

The pure, isolated components were characterized by retardation factor (R_f) values, staining behavior, presence of di- or tetraether, water-soluble hydrolysis products, and molar ratios of phosphate (*P*), sugars, and di- or tetraether (Table 1). The phosphoglycolipids PGL-I and PGL-II were derivatives of the tetraether ($[\alpha]_D$ + 8.7°; reported in (*16*), $[\alpha]_D$ + 7.5°) and contained *P*, hexose, and tetraether in the molar ratio 1:2:1. However, PGL-I contained 1 mole each of glucose and galactose, whereas PGL-II contained 2 moles of galactose. The glycolipids DGT-I and DGT-II also contained the tetraether and hexose (glucose and galactose in DGT-I and galactose only in DGT-II) in the molar ratio 1:2, and could be derived from PGL-I and PGL-II, respectively, by removal of the glycerophosphate moiety with 1*N* methanolic NaOH. The glycolipids DGD-I and DGD-II had the same hexose residues as DGT-I and DGT-II, respectively, but the lipid moiety was the *sn*-2,3-di-*O*-phytanyl diether instead of the tetraether (Table 1).

Complete methylation of PGL-I, DGT-I, or DGD-I yielded 2,3,4,6-tetramethylglucose and 3,5,6-trimethylgalactose, showing the presence of one terminal sugar, glucopyranose, which was linked to galactofuranose at the 2-position (compounds **1**, **2**, and **5** in Fig. 1). Methylation of PGL-II, DGT-II, or DGD-II, however, gave 2,3,5,6-tetramethylgalactose and 2,3,5-trimethylgalactose, showing that the terminal sugar was galactofuranose linked to another galactofuranose at the 6-position (compounds **3**, **4**, and **6** in Fig. 1). The ano-

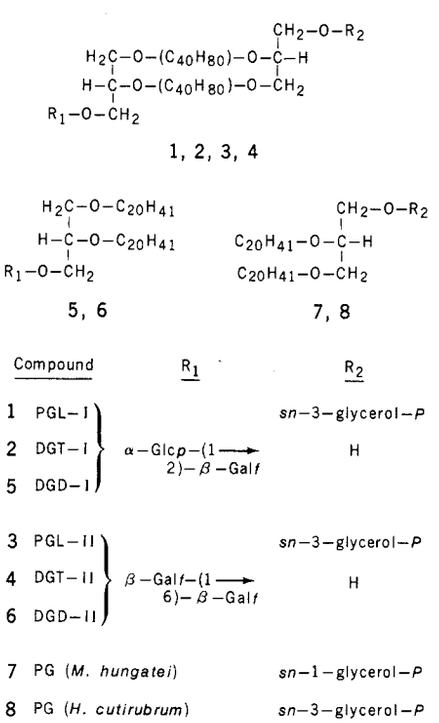

Fig. 1. Structures of complex lipids in *Methanospirillum hungatei*.

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Table 1. Characterization and analysis of complex polar lipids of *Methanospirillum hungatei* (Fig. 1).

Item	PGL-I (1)	PGL-II (3)	DGT-I (2)	DGT-II (4)	DGD-I (5)	DGD-II (6)	PG (7)
TLC, R_f*	0.34	0.46	0.73	0.83	0.68	0.78	0.61
Weight (percent of total lipid)	50	14	0.5	0.2	17	2	5
Molar ratios†							
P/tetraether‡	1	1					
P/diether§							1
Hexose/tetraether	2	2	2	2			
Hexose/diether					2	2	
Hexose/*P*	2	2					

*Thin-layer chromatography (TLC) on silica gel G in a mixture of chloroform, methanol, diethylamine, and water (110:50:8:3.5, by volume). †Lipid-*P* and hexose were determined as described (*9*). ‡Tetraether is 2,3,2′,3′-tetra-*O*-16″,16‴-biphytanyl-di-*sn*-glycerol (*7, 16*). §Diether is 2,3-di-*O*-phytanyl-*sn*-glycerol (*6*).

meric configuration of the sugar residues was established by ^{13}C NMR as α-glucopyranosyl (99.1 ppm) and β-galactofuranosyl (106.7 ppm) in PGL-I; in PGL-II, both residues were β-galactofuranosyl groups (109.0 and 108.4 ppm). These assignments were supported by the ^1H NMR spectra and by molecular rotation data calculated by Hudson's isorotation rule.

In PGL-I and PGL-II, both sugars must be located on one side of the tetraether and the glycerophosphate group on the other (Fig. 1) since, after hydrolysis, completely methylated PGL-I and PGL-II yielded only the free tetraether, whereas DGT-I and DGT-II yielded only the monomethyl tetraether derivative. The glycerophosphate group has the *sn*-3 configuration and is linked to the tetraether by a phosphodiester bond, as shown by the quantitative release of glycerophosphate [59 percent β isomer, 41 percent *sn*-3 isomer (*11, 12*)] by alkaline hydrolysis. The *sn*-3-glycerophosphate residue in PGL-I and PGL-II is enantiomeric with that of the corresponding residue in Gram-positive bacterial phosphoglycolipids (*11, 12*).

The PG component (compound **7** in Fig. 1) was identified as the 2,3-di-*O*-phytanyl-*sn*-1-glycerophosphoryl-*sn*-1′-glycerol, diasteriomeric with that (compound **8** in Fig. 1) found in extreme halophiles (*6*).

On the basis of the above results, the structure of each polar lipid component can be assigned as shown in Fig. 1. The structures of PGL-I and PGL-II suggest that they behave as covalently bonded asymmetric lipid bilayers in methanogen membranes, as was proposed by Langworthy (*7, 17*) for the lipids of thermoacidophiles. Such a structure would impart rigidity and stability to the membranes not possible with a conventional bilayer structure.

Further investigation is required to determine the biosynthetic pathway for these polar lipids, their distribution within the cell and also the orientation of the asymmetric PGL-I and PGL-II within the membranes. Our findings strengthen the concept of a phylogenetic relationship between methanogens, extreme halophiles, and certain thermoacidophiles (*3, 5, 17, 18*).

S. C. KUSHWAHA
M. KATES
Department of Biochemistry
University of Ottawa,
Ottawa, Canada K1N 9B4
G. D. SPROTT
I. C. P. SMITH
Division of Biological Sciences, National Research Council of Canada, Ottawa

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Conversion of 3T3-L1 Fibroblasts to Fat Cells by an Inhibitor of Methylation: Effect of 3-Deazaadenosine

Abstract. *3-Deazaadenosine, an inhibitor of methylation, increased the frequency of conversion of 3T3-L1 fibroblasts to fat cells in a dose-dependent manner. Once converted, the 3T3-L1 fat cells retained their adipose morphology and accumulated triglycerides even when 3-deazaadenosine was removed from the culture medium. 3-Deazaadenosine may perturb cellular methylation and thereby lead to an increase in the frequency of differentiation of 3T3-L1 fibroblasts to fat cells.*

3-Deazaadenosine (3-deaza-Ado), an adenosine analog that is not deaminated or phosphorylated, inhibits methylation reactions mediated by *S*-adenosylmethionine (AdoMet) in vivo or in vitro (*1–4*). 3-Deaza-Ado inhibits biochemical methylations by acting as either an inhibitor or a substrate of *S*-adenosylhomocysteine (AdoHcy) hydrolase (*5–7*). By inhibiting AdoHcy hydrolase with 3-deaza-Ado, the intracellular level of AdoHcy can be increased, and in the majority of cases 3-deaza-AdoHcy is also generated (*1–3, 5, 8*). Thus, the accumulation of AdoHcy or 3-deaza-AdoHcy, or both, leads to perturbation of biochemical methylations that are sensitive to inhibition by these two compounds (*1, 5, 7–9*). The biological effects of 3-deaza-Ado are varied and interesting. It can act as a potent antiviral agent (*8, 10*) and can also reverse the oncogenic transformation induced by Rous sarcoma virus (*8*). It exhibits antimalarial effects in vitro (*11*). Chemotaxis by neutrophils (*12*), phagocytosis by macrophages (*13*), lymphocyte-mediated cytolysis (*3*), capping of membrane immunoglobulin in lymphocytes (*14*), and immunoglobulin E-mediated histamine release from human basophils (*15*) are inhibited by 3-deaza-Ado. It has also been shown that 3-deaza-Ado can inhibit, rapidly and reversibly, synaptic responses between retinal neurons and muscle fibers in culture (*16*).

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