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Lipids from the sulphur-dependent archaebacterium *Thermoproteus tenax*

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The sulphur-dependent archaebacterium *Thermoproteus tenax* has been shown to contain the following ether core lipids: one C_{20},C_{20} glycerol diether and seven C_{40},C_{40} diglycerol tetraethers containing 0–5 cyclopentane rings; a novel tetraether consisting of two glycerols, one acyclic and one bicyclic C_{40} hydrocarbon. The three major glycolipids are built up from one tetraether and 1–3 glucose residues. The main lipid is a phosphoglycolipid consisting of one tetraether, one glucose and one phosphoinositol residue. *T. tenax* lacks fatty acids. The lipids from autotrophically and heterotrophically grown cells differ quantitatively in their contents of the different types of tetraether molecule.

Introduction

Archaebacteria comprise three phenotypic groups, the halophilic, the methanogenic and the sulphur-dependent (formerly thermoacidophilic) archaebacteria [1,2]. The unusual structure of their membrane lipids is a common feature of all archaebacteria; they contain ether lipids built up from isopranoid hydrocarbons and polyols (mostly glycerols) [3,4]. The latter are normally substituted by carbohydrate and/or phosphate groups. The complex lipids from several halobacteria [3,5,6], diverse methanogens [3,4,7–11] and *Thermoplasma acidophilum* [3] have been studied, as well as the lipids from the sulphur-dependent archaebacteria *Sulfolobus* [3,4], *Thermococcus celer* [12] and *Desulfurococcus mobilis* [13].

Here, we describe the structures of the major lipids from *Thermoproteus tenax* [14]. This organism is the main representative of the *Thermoproteales*, the second order of sulphur-dependent archaebacteria [2]. *T. tenax* either grows autotrophically by chemosynthesis of hydrogen sulphide from hydrogen and sulphur, or heterotrophically by sulphur respiration [15]. The analysis was carried out with autotrophically grown cells; however, we also analyzed heterotrophically grown cells in order to study the influence of growth conditions on lipid structure.

Materials and Methods

Materials. All chemicals and solvents used in the lipid analysis were analytical grade.

Growth of the organism. The cells from *T. tenax*, strain Kra-1, Deutsche Sammlung für Mikroorganismen (DSM) 2078, were grown as already described [16].

Chromatography. Analytical thin-layer chromatography (TLC) was carried out in horizontal chambers (HPTLC Linear 28510, Camag) with 10×10 cm HPTLC plates (silica gel 60, F_{254}, Merck). Ether core lipids were separated with chloroform/ethyl acetate (85 : 15, v/v) and glyco- and phospholipids with chloroform/methanol/water (65 : 25 : 4, v/v). Lipids were detected by

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charring with methanol/conc. sulphuric acid (1 : 1, v/v) and heating at 150 °C. Phospholipids were sprayed with the molybdenum blue reagent [17]. GLC analyses were carried out with a Fractovap 2101 gas chromatograph (Carlo Erba), equipped with a flame ionization detector, carrier gas, hydrogen. For GC-MS analyses, the same gas chromatograph (carrier gas, helium) was coupled with the mass spectrometer CH-7A, Varian MAT. Fatty acids and phytanyl chloride were analyzed on a 30 m DB-1 fused silica capillary (J.W. Scientific, Rancho Cordova, CA, U.S.A.) at 100–280 C°/min. C_{40} hydrocarbons were separated on a 10 μm CP™SIL-5 fused silica capillary (Chrompack, Middelburg, The Netherlands) at 150–310 °C, 5 C°/min. For analysis of silylated polyols and carbohydrates, a 25 μm DB-1701 fused silica capillary (J.W. Scientific) at 60–280 °C, 4 C°/min was used. Silylation was performed in MIFL tubes (Antech, Bad Dürkheim, F.R.G.) with 20 μl of Silyl 2110 (mixture of hexamethyldisilazane and trimethylchlorosilane in pyridine). For perfluoropropionylation, glycerol diether (0.1 mg), dissolved in 50 μl of methylene chloride, was derivatized with 50 μl of perfluoropropionic anhydride for 1 h at room temperature. The samples were concentrated to dryness, dissolved in methylene chloride and analyzed isothermally at 300 °C on a 10 μm CP™SIL-5 fused silica capillary.

Physical measurements. Infrared spectra were recorded with the Perkin-Elmer IR 197 spectrometer (Ueberlingen, F.R.G.). Tetraethers were measured between NaCl plates. Optical rotations of tetraethers and glycolipid GL-1, dissolved in chloroform, were obtained with the Perkin-Elmer polarimeter 241-MC. ^{1}H-NMR spectra were measured with 90 and 500 MHz spectrometers (Bruker, Rheinstetten-Forchheim, F.R.G.). Tetraethers, glyco- and phospholipids were dissolved in deuteropyridine and a drop of ^2H_2O. The standard used was tetramethylsilane. ^{13}C-NMR spectra were measured at 23.63 MHz with a Bruker spectrometer; tetraethers and GL-1 were dissolved in C^2HCl_3, PL-3 in $C^2H_3O^2H/C^2HCl_3/^2H_2O$ (60 : 30 : 4, v/v). Mass spectra were recorded with the mass spectrometers CH-7A and MAT-312 coupled with a data system SS200/MS (Varian/Finnigan, Bremen, F.R.G.).

Quantitative analytical methods. Glycerol was quantitatively analyzed with the Test-Combination für die Lebensmittelanalytik (Boehringer, Mannheim, F.R.G.) [18]. For calculation, the content of glycerol from a standard batyl alcohol was measured; we found it to be 76% of the theoretical value. Phosphate contents of phospholipids were determined according to Eibl and Lands [19,20] (reagents from Serva, Heidelberg, F.R.G.). For testing the method, the content of phosphate from a standard fructose 1,6-diphosphate was determined; we found it to be 90% of the calculated value.

Extraction and fractionation of lipids. Extraction and fractionation procedures from Langworthy [21] were modified as described below. Cells were broken in a French press and lyophilized. Lipids were extracted from 25 g lyophilized cells by stirring for 3 h at room temperature twice with 30 ml of chloroform/methanol (2 : 1, v/v) and twice with 30 ml of chloroform/methanol/water (60 : 30 : 4.5, v/v). The lipid extract (1.5 g) was suspended in 250 ml of chloroform and fractionated on a 5 × 15 cm silica gel column (150 g silica gel 60, 0.063–0.200 mm particle size, Merck, Darmstadt, F.R.G.). Neutral lipids were eluted with 3 l of chloroform and polar lipids with 3 l of methanol. The polar lipids (500 mg), dissolved in 50 ml of chloroform/methanol/water (60 : 30 : 4.5, v/v) were further fractionated on a 5 × 22 cm DEAE-cellulose column, prepared with 110 g Servacel DEAE-cellulose 23 SS, analytical grade (Serva) [22]. The column was eluted with 2.5 l of chloroform/methanol (7 : 3, v/v) for glycolipids, 1.3 l of chloroform/acetic acid (3 : 1, v/v) for removal of pigments, 1.3 l of methanol for removal of acetic acid, 2.5 l of chloroform/methanol/33% ammonium/ammonium acetate (70 : 30 : 2 : 0.4, v/v/v/w) for less polar phospholipids and with 2.5 l of chloroform/methanol/water/potassium hydrogen phthalate (60 : 30 : 4.5 : 0.18, v/v/v/w), adjusted to pH 2.5 with conc. HCl, for more polar phospholipids. To remove salts from the phospholipid fractions, the eluates were dried and partitioned between the upper and lower phase from chloroform/methanol/water (2 : 1 : 0.6, v/v).

Elemental sulphur from the culture medium, being extracted into the neutral lipid fraction,

could be removed largely by extraction with hexane. Various lipid fractions were purified by preparative TLC in vertical chambers with 20 × 20 cm plates (0.5 mm layers of silica gel 60, F_{254}, Merck). Diethers and tetraethers were extracted from silica gel with chloroform/methanol (2 : 1, v/v) and glyco- and phospholipids with chloroform/methanol/water (60 : 30 : 4.5, v/v). In order to remove traces of colloidal silica gel, these solutions were extracted with water.

Production of cells without sulphur particles (Rettenberger, M., Max-Planck-Institut für Biochemie, personal communication). Cells without sulphur particles adhered on the surface could be obtained on a small scale by the following procedure: cells were suspended in water and transferred onto a linear gradient from 28% sucrose and 9% glucose to 56% sucrose and 18% glucose, dissolved in a buffer consisting of 10 mM Tris, 1 mM EDTA and 100 mM NaCl, adjusted to pH 7.9 with HCl. The samples were centrifuged into balance for 30 h at 28000 rpm with a SW 28 rotor (Beckmann). After decanting the top layer, a broad zone, consisting of cells and cell fragments without microscopically detectable sulphur particles, was isolated.

Separation of tetraethers. Tetraethers were derivatized with *p*-nitrobenzoyl chloride [23]; the derivatives were isolated from the reaction mixture by preparative TLC, using 10 × 10 cm HPTLC plates (silica gel 60, F_{254}, Merck) and chloroform/ethyl acetate (85 : 15, v/v) as eluent. The mixture of bis-*p*-nitrobenzoyl tetraethers was resolved by HPLC (apparatus from Laboratory Data Control, Riviera Beach, FL 33404, U.S.A.) on a 10 μm Porasil column (3.9 × 300 mm, Waters). The components were eluted isocratically with methylene chloride/acetone (997 : 3, v/v) at a flow rate of 0.7 ml/min, and monitored at 254 nm. After separation, the chromophores were cleaved with 2 M KOH [24].

Lipid degradation procedures. Acidic and alkaline hydrolysis of glyco- and phospholipids, ether cleavages with hydriodic acid and boron trichloride, and reduction of alkyl iodides with zinc/acetic acid were carried out according to Langworthy et al. [21,25,26].

Carbohydrate analysis. Glycolipids, with xylose as the internal standard, were hydrolyzed in screw-cap vials with 1 M methanolic HCl for 16 h at 80 °C. In order to obtain underivatized monosaccharides, the samples were dried and treated with 0.5 M HCl for 13 h at 100 °C. More hydrophilic phosphoglycolipids were hydrolyzed together with the standard using 0.5 M HCl for 20 h at 100 °C. The monosaccharides generated were transformed into the corresponding alditol acetates [27], separated on a 30 m DB-1701 fused silica capillary column (170–270 °C, heat rate: 3 C°/min), and quantitatively estimated by ion selection monitoring at *m/z* 259 and 289 [28]. For sequence analysis, the permethylation method of Hakomori [29] was used; excess amounts of dimethylsulphinyl sodium were assured using a colour reaction with triphenylmethane [30]. Partially methylated alditol acetates were identified by GC-MS [31] on a 30 m DB-1701 capillary (100–250 °C, heat rate: 3 C°/min).

Results

Total lipids from *T. tenax* account for 6% of the cell dry weight. Fractionation of lipids from cells without sulphur particles adhered on the surface yielded the following weights: 5% neutral lipids, 20% glycolipids and 75% phospholipids.

Ether core lipids

The lipid fractions from *T. tenax* were degraded by 1 M methanolic HCl to yield ether core lipids. TLC analysis of these core lipids from glyco- and phospholipid fractions revealed the same bands.

Glycerol diether (Fig. 1A). The less polar chromatographic fraction (R_F 0.8) represents glycerol diether. This fraction, comprising 5% of the core lipids, was cleaved by boron trichloride to yield glycerol and phytanyl chloride. The compounds from *T. tenax* and 2,3-di-*O*-phytanyl-*sn*-glycerol, isolated from *Halobacterium halobium* [32], were compared. On TLC, both substances show identical R_F values; furthermore, the GLC retention times and the mass spectra (direct inlet system) of the perfluoroacetates of both compounds are identical. The configuration of the secondary C-atom of the glycerol moiety is unknown, as we could not measure the optical rotation because of lack of material.

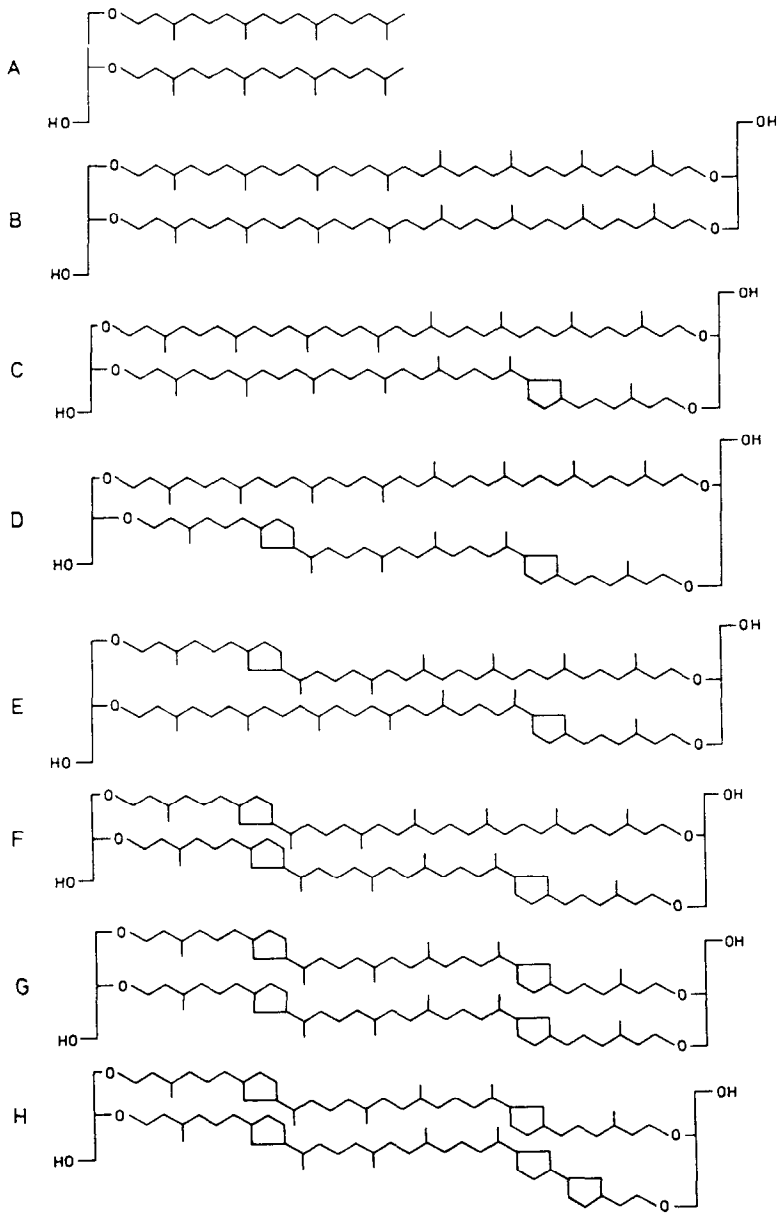

Fig. 1. Structures of the ether core lipids from *T. tenax*. (A) glycerol diether; (B–H) diglycerol tetraether types 0+0–2+3. The 'antiparallel' orientation of glycerols and hydrocarbons has not been proved.

Diglycerol tetraethers (Fig. 1B–H). The second chromatographic fraction (95% of the ether core lipids) is a series of bands (R_F 0.55–0.3), representing several diglycerol tetraethers. Quantitative glycerol analysis after cleavage with boron trichloride and quantitative analysis of C_{40} hydrocarbons after cleavage with hydriodic acid and reduction with zinc/acetic acid, showed that the molar ratio of glycerol to hydrocarbons of these compounds was 1 : 1. An isopranoid acyclic hydrocarbon $C_{40}H_{82}$ (M_r 562), a monocyclic hydrocarbon $C_{40}H_{80}$ (M_r 560), a bicyclic compound $C_{40}H_{78}$ (M_r 558) and, in small amounts, a tricyclic hydrocarbon, $C_{40}H_{76}$ (M_r 556) were identi-

fied by GC-MS. The mass spectra were identical to those of hydrocarbons from *Sulfolobus solfataricus* [33,34].

The tetraether mixture was derivatized to yield bis-*p*-nitrobenzoyl esters and separated by preparative HPLC into five peaks of equal amounts and a small sixth peak. According to fast atom bombardment mass spectrometry analysis, the M_r of the tetraether with the shortest retention time was 1301 ($C_{86}H_{172}O_6$; calculated M_r 1301.31). This tetraether contains the acyclic hydrocarbon $C_{40}H_{82}$ (M_r 562), as shown by GC-MS after degradative procedures. Consequently, this compound consists of two glycerols and two acyclic hydrocarbons; it is a 0 + 0-type tetraether (Fig. 1B). The compound with M_r 1299 ($C_{86}H_{170}O_6$; calc. M_r 1299.29), containing hydrocarbons $C_{40}H_{82}$ and $C_{40}H_{80}$ in equal amounts, is a 0 + 1-type tetraether (Fig. 1C). The substances with M_r 1295 ($C_{86}H_{166}O_6$; calc. M_r 1295.26), 1293 ($C_{86}H_{164}O_6$; calc. M_r 1293.25) and 1291 ($C_{86}H_{162}O_6$; calc. M_r 1291.23) are 1 + 2 (Fig. 1F), 2 + 2 (Fig. 1G) and 2 + 3 (Fig. 1H) -type tetraethers. The component with M_r 1297 ($C_{86}H_{168}O_6$; calc. M_r 1297.28) seemed to be an exception. The molar ratio of the hydrocarbons $C_{40}H_{82}/C_{40}H_{80}/C_{40}H_{78}$ was 1 : 2 : 1. We interpretate this HPLC peak to be an equimolar mixture of the tetraethers 0 + 2 (Fig. 1D) and 1 + 1 (Fig. 1E). This interpretation becomes more clear when considering the tetraethers as macrocycles. The bis-*p*-nitrobenzoyl tetraethers were separable because a cyclopentane ring shortens the hydrocarbon chains. The tetraether 0 + 0 is the largest macrocycle, with the tetraethers 0 + 1, 0 + 2, 1 + 1, 1 + 2, 2 + 2 and 2 + 3 becoming smaller. The tetraethers 0 + 2 and 1 + 1 possess the same number of five-membered rings; therefore, they are of the same size, and were not separable with the applied HPLC system.

The 'orientation' of the glycerols and hydrocarbons of the tetraethers from *T. tenax* remains unknown. De Rosa et al. [35] have shown, by means of ^{13}C-NMR spectroscopy, that the glycerols as well as the isopranoid chains of the 3 + 3-type tetraether from *S. solfataricus* are 'orientated antiparallel'. This tetraether contains two asymmetrically orientated tricyclic hydrocarbons with one cyclopentane ring near the ether bond. This ring, comprising C atoms 3–6 and 17 (Fig. 2), alters the chemical shift of C-atom 1, compared with the chemical shifts of C atoms 1 in the case of the acyclic, monocyclic (ring with C-atoms 7–10 and 18) and bicyclic (rings with C atoms 7–10 and 18 and C atoms 7′–10′ and 18′) hydrocarbons [35]. Therefore, ^{13}C-NMR spectroscopy could solve the problem for the 3 + 3-type tetraether only, but not for the tetraethers from *T. tenax*.

The tetraether species described above, the types 0 + 0, 0 + 1, 0 + 2, 1 + 1, 1 + 2, 2 + 2 and 2 + 3, were found in autotrophically grown cells. In contrast, in heterotrophically grown cells, the tetraethers 1 + 2 and 2 + 2 dominate, and the types 0 + 0 and 0 + 1 are nearly absent.

Neutral lipids

In the neutral lipid fraction from autotrophically grown cells, small amounts of tetraethers 0 + 0 to 2 + 3 were detected on TLC. Furthermore, isopranoid quinones were found in this fraction [16].

Glycolipids

Glycolipids were eluted from the DEAE-cellulose column by a non-ionic solvent, so they lack ionic groups. The organic phase of the methanolic HCl hydrolysate contained tetraethers and small amounts of diether. Glucose was the sole compound identified in the water phase after silylation [36]. The glycolipid fraction reveals six bands on TLC (chloroform/methanol/water (65 : 25 : 4, v/v). The major bands, GL-1, GL-3 and GL-4, were isolated and characterized.

Glycolipid GL-1 (Fig. 3A) (R_F 0.78; 10% of the total lipid weight) is a mixture of two compounds, because it was degraded by methanolic

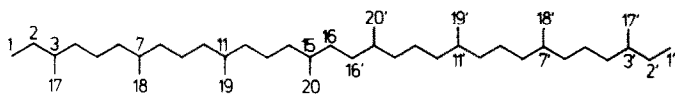

Fig. 2. Numbering of C atoms from the acyclic hydrocarbon $C_{40}H_{82}$.

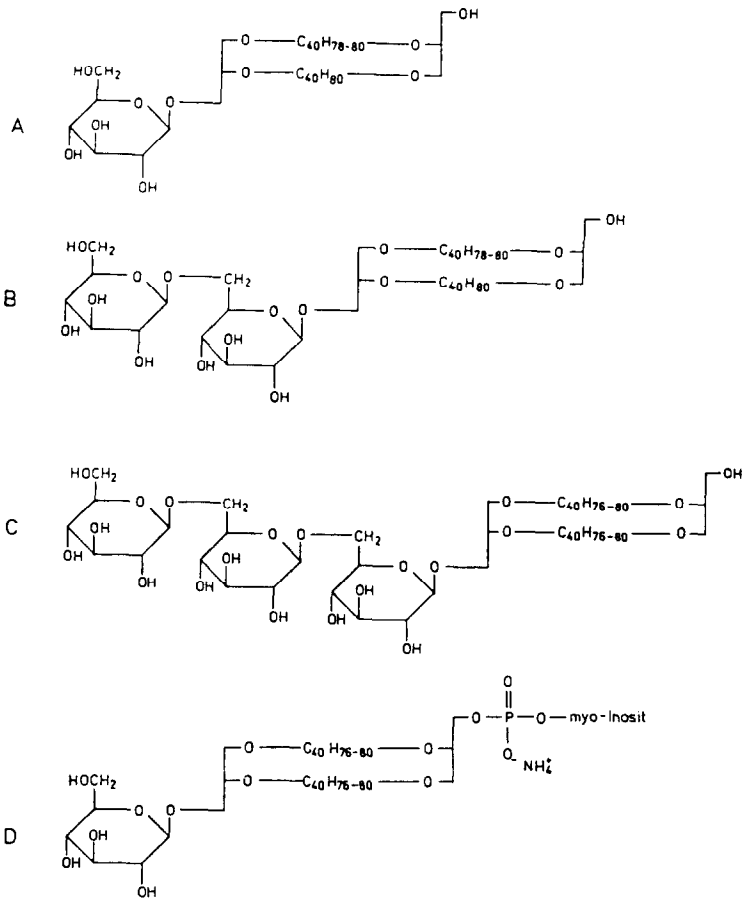

Fig. 3. Structures of the main polar lipids from *T. tenax*. (A) Glycolipid GL-1; (B) GL-3; (C) GL-4; (D) phospholipid PL-3. The β-configuration of all glycosidic linkages from glycolipids GL-3 and GL-4 has not been proved.

HCl to the tetraethers of the 0 + 0 and 0 + 1 type, according to TLC. Quantitative carbohydrate analysis and ^{1}H-NMR integration of protons in the α-position to O atoms (3.6–5.0 ppm) and of protons in the alkyl chains (0.8–2.0 ppm) proved that GL-1 is built up from one tetraether and one glucose residue. A ^{13}C-NMR signal at 103.6 ppm indicates a β-glucosidic linkage [37]. The molecular rotation of +24° (20°C) is in agreement with the molecular rotation of a β-D-glucopyranosyl-diglycerol tetraether (+45°), calculated according to the glycoside rule of Klyne [35,38,39].

Glycolipid GL-3 (Fig. 3B) (R_F 0.75; 2% of the total lipid weight) contains the tetraethers type 0 + 0 and 0 + 1. It is built up from two glucose residues, because partial hydrolysis and TLC analysis proved the presence of GL-1 and tetraethers as reaction products. After methylation and hydrolysis of GL-3, the organic phase contained monomethyl tetraethers (TLC). The carbohydrates in the water phase were reduced and acetylated to yield 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylhexitol (GC-MS) [31]. Consequently, the two glucose residues are attached chain-like with a 1 → 6 linkage to one of the two hydroxyl groups of the tetraethers.

Glycolipid GL-4 (Fig. 3C) (R_F 0.5; 6% of the total lipid weight) is a mixture of lipids containing tetraethers of the 0 + 0 to 2 + 2 type. Quantitative sugar analysis, integration of the ^{1}H-NMR spectrum, partial hydrolysis (degradation products: GL-3, GL-1 and tetraethers) and methylation analysis are consistent with the structure of a

glucopyranosyl- (1 → 6) glucopyranosyl- (1 → 6) glucopyranosyldiglycerol tetraether.

The minor glycolipids GL-2 (R_F 0.76), GL-5 (R_F 0.3) and/or GL-6 (R_F 0.2) might be diether glucosides, since the glycolipid fraction also contains small amounts of glycerol diether. GL-5 and GL-6, having low R_F-values, are probably built up from at least four glucose residues.

Phospholipids

The two phospholipid fractions from autotrophically as well as heterotrophically grown cells reveal six bands on TLC (chloroform/methanol/water (65 : 25 : 4, v/v)): PL-1 (R_F 0.35), PL-2 (R_F 0.3), PL-3 (R_F 0.2), PL-4 (R_F 0.25), PL-5 (R_F 0.1) and PL-6 (R_F 0.07). The main components, PL-3 (Fig. 3D) (R_F 0.2; 50% of the total lipid weight) is an equimolar mixture of lipids containing the tetraether types 0 + 0 to 2 + 2. From the water phases of hydrolyses with 1 M methanolic HCl and 6 M HCl, respectively, glucose and *myo*-inositol were identified by silylation and GC-MS [40]. Quantitative sugar analysis, phosphate assay and integration of the ^{1}H-NMR spectrum showed that PL-3 is built up from one tetraether, one glucose, one inositol and one phosphate group. The structure of PL-3, having phosphoinositol and β-D-glucopyranosyl residues at each side of the core lipid, is confirmed by following facts: PL-3 was degraded to GL-1 by alkaline hydrolysis (TLC); methylation analysis yielded underivatized tetraethers and, after reduction and acetylation, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol; the ^{13}C-NMR spectrum revealed a signal at 61.6 ppm, assigned to the 'free' C-6 atom of glucose [37], but there was no resonance at 63.1 ppm, assigned to the 'free' glycerol residue of tetraether [35]; a resonance at 103.6 ppm indicates a β-glucosidic linkage [37].

Using the methods described above, phospholipid PL-5 (R_F 0.1; 14% of the total lipid weight) was shown to be built up from one tetraether, three glucoses, one inositol and one phosphate. Besides the phosphoglycolipids PL-3 and PL-5, there are small amounts of phospholipids without sugar groups. Phospholipid PL-2 (R_F 0.3) is degraded to a glycerol diether by alkaline hydrolysis.

Discussion

The lipids from *T. tenax* are typical archaebacterial ether lipids. We could not find any extra, or any different fatty acid methyl esters in a control experiment of the same apparative size, i.e., using the same equipment, volumes and purities of solvents, isolation techniques and analyses by GLC and GC-MS. Kates et al. [41,42] showed that in *Halobacterium cutirubrum*, a fatty acid synthetase biosynthesizes palmitic acid at a low level under non-physiological conditions. The cells of the closely related *Halobacterium halobium*, grown on a synthetic medium free from fatty acids, however, did not contain fatty acids (Schäfer, W. and Oesterhelt, D., unpublished data). So the question as to whether *H. cutirubrum* is the only archaebacterium capable of biosynthesizing fatty acids remains unsolved.

The ether core lipids from *T. tenax* are, like the lipids from *Sulfolobus* [4], mainly tetraethers (95%) and, in small proportions, diethers (5%). *T. tenax* is, besides *Methanosarcina barkeri* [43], the second anaerobic archaebacterium to contain cyclopentane rings in the alkyl chains of the tetraether lipids. Obviously, the unknown biosynthesis of these rings works without oxygen. The ether core lipids from *T. tenax*, with the exception of the tetraether 0 + 2, were already found in *S. solfataricus* [4] and *T. acidophilum* [44]. Until now, tetraethers from $n + n$ and $n + (n + 1)$ type, but no molecules from the $n + (n + 2)$ type [4,44], like the novel tetraether 0 + 2, have been known.

The lipids from *T. tenax* consist of 5% neutral lipids, 20% glycolipids and 75% phospholipids, a distribution typical of archaebacteria [3,4]. The neutral lipid fraction contains, like other archaebacteria, small amounts of unbound tetraethers, which are probably biosynthetic precursors of glyco- and phospholipids.

The polar lipids from *T. tenax*, with the exception of diglucosyl tetraether [11], are novel structures [3–13]. The glycolipids amount to 20% of the total lipid weight; they are probably not only biosynthetic precursors of the phosphoglycolipids, but may fulfill, as non-ionic lipids, specific functions in the cell membrane. The polar tetraether lipids from archaebacteria have a common building plan: the carbohydrates of glycolipids are lin-

ked to only one of the two hydroxyl groups of the tetraethers; the phosphate groups of the phosphoglycolipids are bound to the second hydroxyl group [4,7,11].

The lipids from heterotrophically grown cells of *T. tenax* closely resemble those from autotrophically grown cells. Glycolipids and phospholipids from both cell types are identical. The only difference is the number of cyclopentane rings of the tetraethers. Autotrophically grown cells produce equal amounts of the tetraethers 0 + 0 to 2 + 2, and the heterotrophically grown cells, mainly the types 1 + 2 and 2 + 2. Obviously, the number of cyclopentane rings depends on nutrition conditions, and especially on the sources of the carbon and hydrogen atoms. The analysis of the lipids from *Sulfolobus acidocaldarius* yielded conflicting results. Here, autotrophically and heterotrophically grown cells contain the same number of cyclopentane rings; the structures of their polar lipids, however, are partially different [45]. The number of five-membered rings of the lipids from *Sulfolobus* increases when the temperature at which cells are grown is raised [46].

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