A C₂₅,C₂₅ Diether Core Lipid from Archaeobacterial Haloalkaliphiles

By M. DE ROSA,¹ A. GAMBACORTA,¹ B. NICOLAUS¹ AND W. D. GRANT^{2*}

¹*Istituto di Chimica di Molecole di Interesse Biologico, C.N.R., via Toiano 6, Arco Felice, Napoli, Italy*

²*Department of Microbiology, School of Medicine and School of Biological Sciences, University of Leicester, Leicester LE1 7RH, U.K.*

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The membrane lipids from an archaeobacterial haloalkaliphile were shown to be based almost entirely on diethers containing C₂₅ isopranyl chains. The 'universal' C₂₀,C₂₀ archaeobacterial diether core lipid (2,3-di-*O*-phytanyl-*sn*-glycerol) made up only 9% (w/w) of the total isopranoid diether fraction. The rest of the isopranoid diether fraction comprised 85% (w/w) C₂₀,C₂₅ diether (2-*O*-sesterterpanyl-3-*O*-phytanyl-*sn*-glycerol) and 6% (w/w) of a novel C₂₅,C₂₅ diether (2,3-di-*O*-sesterterpanyl-*sn*-glycerol).

INTRODUCTION

One of the key distinguishing features of archaeobacteria is the presence of a cell membrane containing unusual ether-linked isopranyl lipids. All archaeobacteria whose lipids have so far been examined possess membranes based on or derived from a 'universal' C₂₀,C₂₀ diphytanyl diether glycerol core lipid (Kates, 1976; De Rosa *et al.*, 1977*a*; Langworthy, 1977; Langworthy *et al.*, 1982). Thus, certain of the methanogenic archaeobacteria have a membrane bilayer almost exclusively composed of this universal core lipid, whereas others possess a mixed bilayer containing different proportions of both C₂₀,C₂₀ core lipids and 'head to head' linked C₄₀,C₄₀ bidiphytanyl derivatives (Balch *et al.*, 1979; Langworthy *et al.*, 1982). Thermoacidophilic archaeobacteria have membranes containing mainly partially cyclized C₄₀,C₄₀ core lipids (De Rosa *et al.*, 1977*a*; Langworthy *et al.*, 1982). The majority of halophilic archaeobacteria have been shown to possess only C₂₀,C₂₀ core lipids (Kates, 1976; Ross *et al.*, 1981), but the phenotypically distinct archaeobacterial haloalkaliphiles have an additional core lipid in substantial amounts; this particular lipid is an asymmetric diether with one C₂₀ phytanyl chain and one C₂₅ sesterterpanyl chain (De Rosa *et al.*, 1982). The implications of this unusual lipid for membrane structure were discussed by De Rosa *et al.* (1982) who suggested that haloalkaliphiles may have a 'zip' membrane which might be thicker than other archaeobacterial membrane types.

We report here that a newly isolated haloalkaliphile has lipids largely based on the C₂₀,C₂₅ core diether and additionally has small amounts of both a novel C₂₅,C₂₅ (disesterterpanyl) core diether and C₂₀,C₂₀ (diphytanyl) core diether lipids.

METHODS

Isolation and cultivation of haloalkaliphiles. Samples of solar evaporation pond liquor from Lake Magadi, Kenya were supplemented with 0.01% (w/v) peptone (Oxoid), 0.1% (w/v) glucose and 0.1% (w/v) KNO₃ and incubated statically at 37 °C in the light (daylight fluorescent strip lighting 750 lx) until turbidity occurred. These enrichments were subcultured on the agar medium of Tindall *et al.* (1980), and colonies selected and recultured on agar media under the same conditions. Strains were then grown in liquid medium as previously described (Tindall *et al.*, 1980) and cells harvested in the early-stationary phase of growth and freeze dried.

Extraction, purification and characterization of diethers. Freeze-dried cells were initially screened for novel diether lipids by the procedures of Ross *et al.* (1981). Cells were then extracted, hydrolysed with methanolic HCl, and the lipid methanolsate was chromatographed on silica gel columns as described by De Rosa *et al.* (1982). The diether fraction so obtained was further resolved by high performance liquid chromatography (HPLC) using Microporasil

columns (De Rosa *et al.*, 1982). Molecular weight determinations were performed by vapour pressure osmometry in chloroform (De Rosa *et al.*, 1982). Infrared spectra of diethers and optical rotations were recorded as previously described (De Rosa *et al.*, 1982). Acetylation and BCl_3 cleavage of ethers was carried out using the procedures of De Rosa *et al.* (1980). Isopranoid iodides and acetates were produced by the methods of De Rosa *et al.* (1977a). Gas chromatography/mass spectrometry of diether acetates and other GLC procedures were as described by De Rosa *et al.* (1982). ^1H -NMR, ^{13}C -NMR and mass spectra were recorded as described by De Rosa *et al.* (1977a, b).

RESULTS AND DISCUSSION

A number of isolates exhibiting characteristic colony morphologies were analysed for novel diether core lipids by thin-layer chromatography using the double development system of Ross *et al.* (1981). One particular strain, designated SP8, had relatively minor amounts of the 'universal' $\text{C}_{20}, \text{C}_{20}$ core diether, a very large proportion of the $\text{C}_{20}, \text{C}_{25}$ core diether, and unusually, a diether moiety chromatographically distinct from both $\text{C}_{20}, \text{C}_{20}$ and $\text{C}_{20}, \text{C}_{25}$ moieties (Fig. 1).

The total complex lipid content of SP8 was about 8% (w/w) and hydrolysis gave a glycerol isopranoid ether fraction. Analysis by HPLC showed that 2-*O*-sesterterpanyl-3-*O*-phytanyl-*sn*-glycerol, 2,3-di-*O*-phytanyl-*sn*-glycerol and the unknown represented 85%, 9% and 6% (w/w) of the mixture, respectively. The optical rotation ($[\alpha]_D \simeq 7.3$) and infrared spectrum of the unknown were almost identical to those of the well characterized 2,3-di-*O*-phytanyl-*sn*-glycerol (Kates, 1976; Ross *et al.*, 1981). Hydrolysis of the unknown with HI, followed by conversion of the alkyl iodides into alkyl acetates gave a single species of isopranoid acetate whose GLC retention time and gas chromatography/mass spectrometry cleavage pattern were identical to those of the sesterterpanyl acetate previously described (De Rosa *et al.*, 1982).

^1H -NMR spectra of the monoacetylated diether indicated a signal due to CH_2OAc protons ($\delta 4.14$) coupled with a single CHO proton signal ($\delta 3.5$), coupled and overlapping with a single

Fig. 1. Thin-layer chromatographic analysis of whole-organism methanolysates of (right hand track) SP8 and (left hand track) a 'control' haloalkaliphile which contains roughly equal proportions of $\text{C}_{20}, \text{C}_{20}$ and $\text{C}_{25}, \text{C}_{20}$ core diether moieties. The unknown diether moiety is arrowed. Plates were developed and redeveloped using the procedures of Ross *et al.* (1981).

CH_2O signal, this pattern being characteristic of a γ acetate of an α, β glycerol diether (De Rosa *et al.*, 1977a, 1982). Mass spectra of the acetylated diether showed a peak at m/e 775 (loss of the acetyl group); at m/e values higher than 250 there were two peaks associated with the loss of aliphatic chains linked to the glycerol carbons at the α and β positions, and peaks at m/e 453 and 467 are related to the loss of $-CH_2-O-C_{25}H_{51}$ and $-O-C_{25}H_{51}$, respectively (Fig. 2a). ^{13}C -NMR spectra of the C_{25}, C_{25} diethers (De Rosa *et al.*, 1982) confirmed regular head to tail isoprenoid chains. Vapour pressure osmometry of the new diether gave a molecular weight of 790 ($C_{53}H_{108}O_3 = 792$). These results establish the structure of the new diether lipid as that shown in Fig. 2(b) with two regular 2,6,10,14,18-pentamethyleicosane (sesterterpane) chains located on the α and β glycerol carbon atoms.

Four major types of lipid domain are currently considered to occur in archaeobacterial membranes (De Rosa *et al.*, 1982). The simplest type composed exclusively of C_{20}, C_{20} core lipids occurs in many halophiles and certain methanogens (Fig. 3a), whereas a second type of membrane containing mixtures of C_{20}, C_{20} and C_{40}, C_{40} core lipids is to be found in other methanogens. The membrane structure proposed for thermoacidophiles has been considered to be a further distinct type because of a very high proportion of C_{40}, C_{40} core lipids, which are usually partially cyclized (De Rosa *et al.*, 1982). The fourth type of membrane we have proposed is the 'zip' structure, which has roughly equal proportions of asymmetric C_{20}, C_{25} core lipids as well as C_{20}, C_{20} core lipids (Fig. 3b). In the 'zip' membranes we suppose that alkyl chains located in different faces of the lipid bilayer penetrate into the opposite half of the lipid bilayer, and there are implications for the thickness of the membrane (Fig. 3a, b). From the results reported here we can suggest a further 'zip' type of haloalkaliphile membrane predominantly composed of C_{20}, C_{25} core lipids, but with a small proportion of both C_{20}, C_{20} and C_{25}, C_{25} core lipids (Fig. 3c). The halophilic phenotype thus may encompass examples of three different classes of archaeobacterial membrane, the exclusively C_{20}, C_{20} bilayer type exemplified by *Halobacterium halobium* (Kates, 1976), a 'zip' type membrane composed of roughly equal proportions of C_{20}, C_{20} and C_{20}, C_{25} core lipids, exemplified by Lake Magadi haloalkaliphilic isolate MS3 (De

Fig. 2. (a) Structure of the acetylated C_{25}, C_{25} glycerol diether indicating the characteristic mass spectral cleavage pattern due to the presence of C_{25} isoprenoids on the C2 and C3 of glycerol (see text). (b) Structure of new C_{25}, C_{25} diether core lipid.

Fig. 3. Halophile membrane structures. (a) Bilayer membrane structure formed by C_{20}, C_{20} diether lipids proposed for *Halobacterium* spp. (and some methanogens) (Kates, 1976; Langworthy *et al.*, 1982). (b) 'Zip' type 1 membrane formed by C_{20}, C_{25} and C_{20}, C_{20} diethers proposed for certain haloalkaliphiles (De Rosa *et al.*, 1982). (c) 'Zip' type 2 membrane proposed for haloalkaliphile strain SP8. C_{25}, C_{25} and C_{20}, C_{20} diethers are paired among a majority of C_{20}, C_{25} diethers.

Rosa *et al.*, 1982), and the present mixed bilayer in strain SP8, largely composed of C_{20}, C_{25} core lipids with small amounts of C_{20}, C_{20} and C_{25}, C_{25} core lipids. The structure of this novel membrane displayed in Fig. 3(c) is only one of the possible interpretations, but seems to us the most plausible. Whether this membrane type really is thicker than the type displayed in Fig. 3(a) remains to be established.

C_{25} isopranoids including 2,6,10,14,18-pentamethyleicosane have been reported as constituents of oils, shales and sediments (Brassell *et al.*, 1981; Langworthy *et al.*, 1982) and in particular have been shown to be present in significant quantities in sediments representing lagoonal-type saline environments (Waples *et al.*, 1974). It is becoming widely acknowledged that many of these isopranoids may derive in part at least from the membranes of archaebacteria. Prior to the discovery of the haloalkaliphiles it had always been something of a mystery that C_{25} isopranoids sometimes comprised a significant proportion of the isopranoid fraction of sediments and oils when C_{25} isopranoids were present only in very small amounts as neutral lipids in a restricted number of thermoacidophilic and methanogenic archaebacteria (Holzer *et al.*, 1979; Tornabene *et al.*, 1979; Langworthy *et al.*, 1982). The realization that haloalkaliphilic archaebacteria have cell membranes containing large amounts of C_{25} isopranoid ethers adds another dimension to the evidence linking archaebacterial metabolism to geochemical fossil formation.

We have yet to characterize extensively the complex lipids of SP8 or indeed those of any of the haloalkaliphiles, although this work is in hand. Our preliminary results (H. N. M. Ross & W. D. Grant unpublished) suggest that equivalent complex lipids based on different core lipids are to be found in this group. The taxonomic and phylogenetic position of SP8 within the haloalkaliphiles and the relationship of the haloalkaliphiles to the rest of the halophiles will be published at a later date.

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