System. Appl. Microbiol. 7, 278–285 (1986)

Lipid Biogenesis in Archaebacteria*

MARIO DE ROSA[1] and AGATA GAMBACORTA

Istituto per la Chimica di Molecole di Interesse Biologico, C.N.R., 80072 Arco Felice, Napoli, Italy

Summary

Archaebacteria, which at present comprise three phenotypes including halophiles, methanogens and thermophiles, have lipids characterized by unusual structural features that can be considered as specific taxonomic markers. Whereas all other hitherto known living organisms have membrane lipids based on ester linkages, all archaebacteria studied up to the present possess lipids based on ether linkages formed by the condensation of glycerol or more complex polyols with isoprenoid alcohols of 20, 25 or 40 carbon atoms. This presentation surveys the most important aspects of archaebacterial lipid biosynthesis, dealing in particular with the pathway of isoprenoid assembly, the mechanism of ether linkage formation and with the biogenetic origin of alcohols of low molecular weight.

Key words: Archaebacteria – Ether lipids – Isoprenoid lipids – *Sulfolobus solfataricus* – Lipid biosynthesis – Bipolar lipids – Thermophilic bacteria

Introduction

Archaebacteria which at present comprise a considerable variety of methanogenic, halophilic and thermophilic species, have been separated from the classically recognized bacteria on grounds which imply a prolonged evolutionary separation (*Balch* et al., 1977; *Woese* and *Fox*, 1977; *Woese*, 1982). All these microorganisms thrive in environments that would normally kill any other known organism; they are now isolated in a few peculiar ecological niches, which include stagnant water, the rumen of animals and thermal volcanic environments for methanogens (*Zeikus*, 1977; *Zeikus* et al., 1980), saturated brine for extreme halophiles (*Gaber* and *Truper*, 1982; *Javor* et al., 1982; *Tindall* et al., 1984) and thermal environments for extreme thermophiles (*De Rosa* et al., 1986 a).

In view of the key role of the membrane in the ability of the archaebacteria to survive in the face of drastic environmental stresses, extensive studies have been undertaken to characterize the structural identity and the biogenetic origin of the membrane lipids (*Kates* et al., 1970; *Langworthy* et al., 1982; *De Rosa* et al., 1982 b; *Bu'Lock* et al., 1983; *De Rosa* et al., 1986 a).

All the hitherto identified membrane lipids of the archaebacteria are characterized by unusual structural features that can be considered as specific taxonomic markers of this group of microorganisms. Whereas all other living organisms thus far investigated have membranes based on ester linkages formed by the condensation of alcohols and fatty acids, the archaebacteria have lipids based on ether linkages. These molecules are obtained by the condensation of glycerol or more complex polyols (nonitol or tetritol) with isoprenoid alcohols of 20, 25 or 40 carbon atoms. Moreover, it is worth noting that all glycerol ethers in archaebacteria contain a 2,3-di-O-*sn*-glycerol, which is unusual since the glycerol in the naturally occurring glycerophosphatides or diaclyglycerols is known to have a *sn*-1,2 stereochemistry (*Kates* et al., 1970; *De Rosa* et al., 1977 a; *Langworthy* et al., 1982; *Bu'Lock* et al., 1983).

Although isoprenoids with specific functions occur in the lipid membranes of most cells (sterols as fluidization agents, prenylated quinones as redox carriers, prenols as glycosylation coenzymes), the ether lipids dealt with here are the only such compounds that provide the major structural components of the membrane in which they occur.

This presentation is meant to be a survey of the most important aspects of archaebacterial lipid biosynthesis, dealing in particular with the pathway of isoprenoid assembly, the mechanism of ether linkage formation and the biogenetic origin of alcohols of low molecular weight (glycerol, nonitol).

Biogenetic studies have been performed primarily with *Sulfolobus solfataricus*, a member of the thermophilic ar-

* Paper given at the International Workshop on Biology and Biochemistry of Archaebacteria, Munich-Martinsried, June 27 to 30, 1985.

[1] Author to whom correspondence should be addressed.

Abbreviations used GDGT glycerol-dialkyl-glycerol tetraethers
GDNT glycerol-dialkyl-nonitol tetraethers
NMR nuclear magnetic resonance

chaebacteria group (*De Rosa* et al., 1975; *Zillig* et al., 1980) since the lipids of this microorganism are characterized by a large variety of structural types in which the most important features of archaebacterial lipids are represented.

Lipid Structure of Archaebacteria

The basic structural elements of all complex lipids present in the halophilic archaebacteria are depicted in Fig. 1. The membrane lipids of these microorganisms derive from the structures of Fig. 1, the free –OH group of which is linked with sugars, phosphoric residues, etc. (*Kates* and *Kushwaha*, 1978; *Kushwaha* et al., 1982; *Ross* and *Grant*, 1985), giving rise to a large range of molecules. All extreme halophilic archaebacteria possess lipids based on 2,3-di-0-phythanyl-*sn*-glycerol (Fig. 1 a). The extremely alkaliphilic halophiles living at pH 10 (*Tindall* et al., 1984) and some strains of neutral halophiles (*Ross* and *Grant*, 1985) also have lipids based on the diethers of Fig. 1 b and 1 c, in which one or two isoprenoid chain(s) are of sesterterpanilic nature (*De Rosa* et al., 1982 a and *De Rosa* et al., 1983 a).

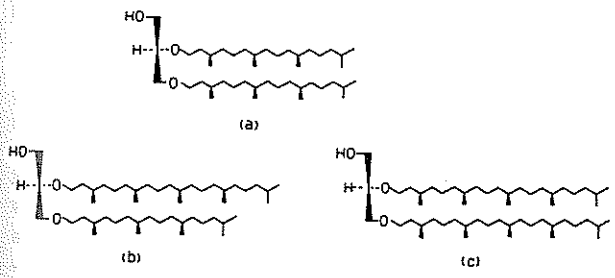

Fig. 1. 2,3-di-0-phytanyl-*sn*-glycerol, the basic component of membrane lipids of all halophilic archaebacteria (a); 2-0-sesterterpanyl-3-0-phytanyl-*sn*-glycerol (b) and 2,3-di-0-sesterterpanyl-*sn*-glycerol (c), basic components of some membrane lipids of halophilic archaebacteria.

The situation in methanogenic archaebacteria appears to be more complex. In most cases the membrane lipids are based on the diphytanyl-glycerol-diether (Fig. 1 a) and the di-biphytanyl-diglycerol-tetraether (Fig. 2 a), the latter being formed by dimerization of two diphytanyl-glycerol-diethers where head-to-head linkage between the terminal methyls occurs (*Makula* and *Singer*, 1978; *Kushwaha* et al., 1981 a; *Kushwaha* et al., 1981 b; *Langworthy* et al., 1982). In addition, lipids of *Methanococcus jannaschii* are based on the macrocyclic diether of Fig. 2 b, which is formed by the head-to-head linkage between the terminal methyls of the two phytanyl residues present in the 2,3-di-0-phytanyl-*sn*-glycerol (Fig. 1 a) (*Comita* and *Gagosian*, 1983; *Comita* et al., 1984). In the *Methanolobus* species, in addition to the diphytanyl-glycerol-diether (Fig. 1 a), 2-0-sesterterpanyl-3-0-phytanyl-*sn*-glycerol (Fig. 1 b) is also present (*Grant* et al., 1985). Finally in Fig. 2 c and d two new structural types found to be present in *Methanosarcina barkeri* are reported; the first is the tetritol-diphytanyl-diether of Fig. 2 c, the second is the 3-0-phytanyl-*sn*-glycerol (Fig. 2 d). In *Methanosarcina* di-biphytanyl-diglycerol-tetraethers with cyclopentane rings in the isoprenoid C_{40} chains are also present, as depicted in Fig. 3 c, d, h (*De Rosa* et al., 1986 b). The complex lipids of methanogens stem from the interaction of the free -OH function(s) with sugars, polyols, phosphoric residues, etc. (*Kushwaha* et al., 1981 b; *Makula* and *Singer*, 1978; *Grant* et al., 1985; *Comita* et al., 1984).

The thermophilic microorganisms have complex lipids based mainly on the structural unities included in Fig. 3, and on the 2,3-di-0-phytanyl-*sn*-glycerol of Fig. 1 a; the various structural components may be present in differing ratios, depending on the microorganism. The available data on the lipid composition of the genera *Desulfurococcus*, *Thermoproteus*, *Thermofilum* and *Pyrodictium* indicate the occurrence of ether lipids based on C_{20} phytanyl chains and on C_{40} biphytanyl chains, without details of their fine structure being available (*Zillig* et al, 1981; *Zillig* et al., 1982; *Stetter* et al., 1983; *Zillig* et al. 1983). In *Thermococcus*, however, complex lipids are based solely on

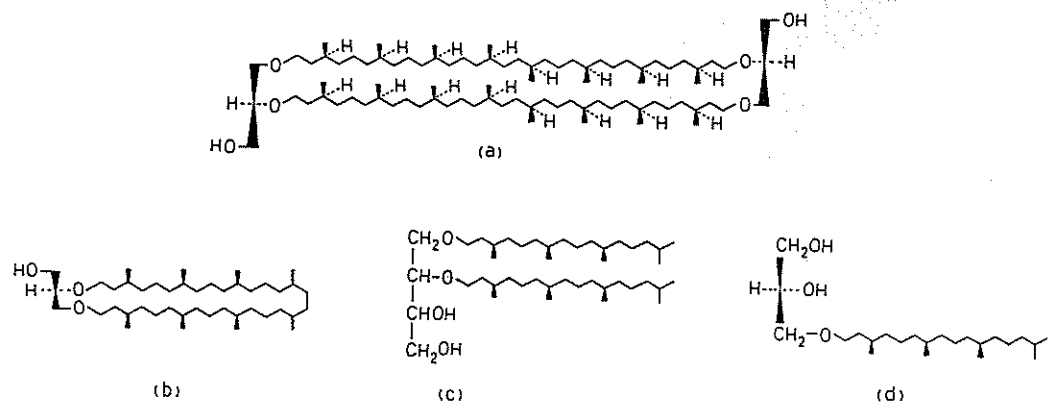

Fig. 2. Glycerol-dialkyl-glycerol tetraether, the basic component of membrane lipids of some methanogenic archaebacteria (a); macrocyclic diphytanyl-diether, the basic component of membrane lipids of *Methanococcus jannaschii* (b); tetritol-diphytanyl-diether (c) and 3-0-phytanyl-*sn*-glycerol (d), basic components of membrane lipids of *Methanosarcina barkeri*.

2,3-di-0-phytanyl-*sn*-glycerol (Fig. 1 a) (*De Rosa* et al., 1986 c.

More extensive studies have been carried out on lipids of the *Sulfolobus* species, which are based essentially on tetraethers shown in Fig. 3 that can be divided into two structural categories (*Langworthy* et al., 1982; *De Rosa* et al., 1983 b). The first type, R=H, designated as glycerol-dialkyl-glycerol-tetraethers (GDGT, Fig. 3 a–i) is formed by two *sn*-2,3-glycerol moieties bridged by two isoprenoid C_{40} diols, via ether linkages, formally derived from the ω,ω' linkages of two 0-phytanyl residues. In the second group, R=$C_6H_{13}O_6$, named glycerol-dialkyl-nonitol tetraethers (GDNT, Fig. 3 a'-i'), a branched nonitol, called calditol, replaces one of the glycerines.

The C_{40} components in these lipids differ in the additional feature of up to four cyclopentane rings; the degree of cyclization in the biphytanyl components is sensitive to environmental parameters such as temperature (*De Rosa* et al., 1980 a). 2,3-di-0-phytanyl-*sn*-glycerol (Fig. 1 a) (*Langworthy* et al., 1982; *De Rosa* et al., 1983 b) and the glycerol-trialkyl-glycerol-tetraether of Fig. 3 e (*De Rosa* et al., 1983 b) are present as minor components in *Sulfolobus*.

Thermoplasma complex lipids are based both on 2,3-di-0-phytanyl-*sn*-glycerol (Fig. 1 a) and on tetraethers a, b, and c of Fig. 3 (*Langworthy*, 1979).

The complex lipids of the thermophilic archaebacteria, which have been extensively studied only in the genus *Sulfolobus*, stem from the ethers in Fig. 1 a and 3, the free -OH functions of which are linked to other molecular groups such as sugars and/or inositol phosphate or to other compounds (*Langworthy* et al., 1972; *Longworthy* et al., 1974; *Langworthy*, 1977; *De Rosa* et al., 1980 b).

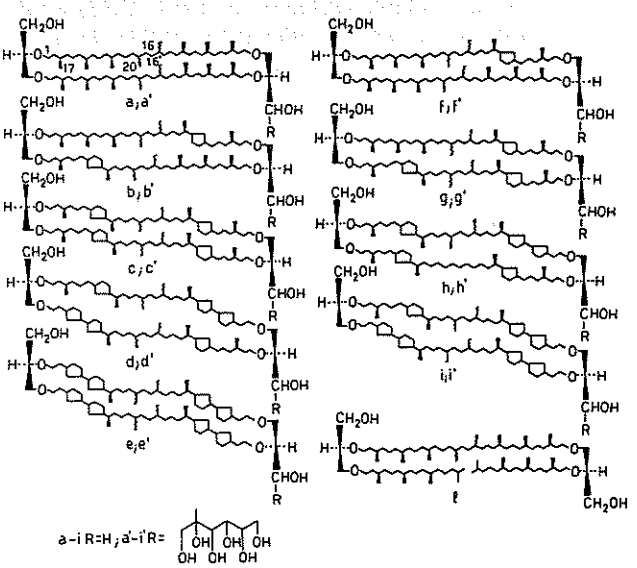

Fig. 3. Structural types of isoprenoid ethers, the backbone of complex lipids of thermophilic archaebacteria.

Isoprenoid Biosynthesis in Archaebacterial Lipids

The isoprenoid chains of the archaebacterial ether lipids are efficiently and selectively labelled subsequent to the uptake of either labelled acetate or labelled mevalonate by *Halobacterium* (*Kates* et al., 1978), *Thermoplasma* (*Langworthy* et al., 1972) and *Sulfolobus* (*Langworthy* et al., 1974; *De Rosa* et al., 1977 b).

In *Sulfolobus solfataricus* the dilution values (specific activity of precursor/specific activity of product) of [1–^{14}C]-acetate and [2–^{14}C]-mevalonate in the glycerol moiety of GDGT (Fig. 3 a–i) are 2.5×10^5 and 5.6×10^5, respectively, whereas they are 7.2×10^3 and 1.2×10^3, respectively, in the isoprenoid moiety. The incorporation of mevalonate is particularly selective for the isoprenoid chain, as could be expected for a conventional isoprenoidic pathway.

The biosynthetic origins of the individual carbon atoms of the C_{40} chain were deduced from ^{13}C NMR studies of the biosynthetically enriched C_{40} derivatives. Comprehensive studies of the labelling patterns from [1–^{13}C]- or [2–^{13}C]-acetate in the biphytanyl components of *Sulfolobus* lipids (*De Rosa* et al., 1977 b) have been informative, since they establish the overall applicability of the classic pathway from acetate to isoprene units. The experiments show that the individual isoprene units are formed by way of mevalonate and are labelled via acetate in the normal fashion m_2c-mc (m=methyl, c=carboxyl). In particular, data on enrichment with [2–^{13}C] acetate confirm, on a biogenetic basis, the localization and dimension of the cycles leading to the C_{40} isoprenoid chain. Cyclopentane rings originate via C-C coupling between methyls 17,17'; 18,18' and methylenes 6,6'; 10,10', respectively (for numbering see Fig. 4 a). Moreover the analysis of ^{13}C NMR data unambiguously indicate that the cyclopentane rings are 1,3 trans substituted, although the mutual stereochemistry of the vicinal rings (Fig. 4 d, e) remains unknown. These results do not give an *a priori* indication of stereochemical constraints at the C_5 pyrophosphate or prenyltransferase stage; there is equally no indication as to which two of the four possible methyl groups (in two presumed tetraprenyl intermediates) are utilized in forming the unique 16,16' head-to-head linkage (for numbering see Fig. 4 a).

To clarify these biogenetic problems, studies of [1,2–^{13}C]-acetate incorporation into the lipids of *S. solfataricus* (*De Rosa* et al., 1980 c) have been carried out. It is well known that this label allows the carbons derived from C-2 of mevalonate to be distinguished from those from C-3' by observation of the ^{13}C–^{13}C couplings in "intact" acetate residues. In all C_{40} isoprenoids (Fig. 4) the signals from carbons 4,4'; 8,8'; 12,12'; 16,16' all appear as singlets of enhanced intensities; the remaining 32 carbons all give rise to signals with satellite pairs due to ^{13}C–^{13}C couplings (J=*ca* 35 Hz). The paired and unpaired carbons are located as shown in Fig. 4. The unpaired carbon atoms are those which derive from C-2 of mevalonate and their "in chain" location in the C_{40} isoprenoid shows that the assembly of the tetraprenyl components has followed the normal pattern, as, for example, evidenced in

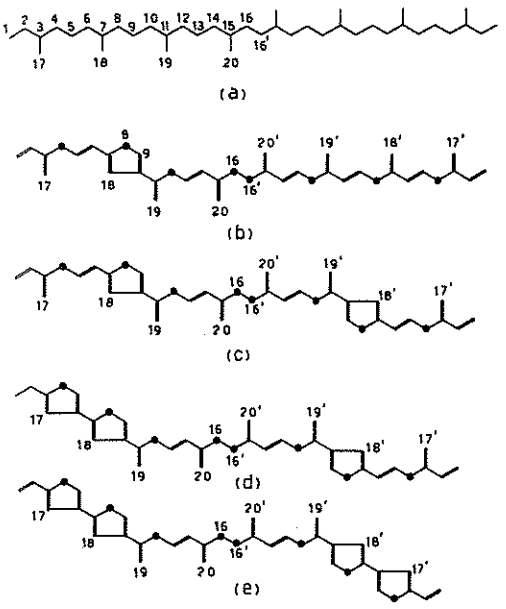

Fig. 4. Labelling pattern of C_{40} isoprenoid biogenetically enriched with 1,2–^{13}C acetate. In the symbology adopted the tract marked represents a situation of C–C coupling in the ^{13}C spectrum. The closed circles (●) indicate the carbons originating from C–2 of a mevalonate intermediate.

the formation of all-transgeranylgeraniol. The realtionship between the structures with $CHCH_3$ and those with cyclopentane rings is confirmed, while the fact that the signals from the central carbons 16 and 16′ are also unpaired shows that the head-to-head coupling reaction (whatever its mechanism) occurs with sufficient stereospecificity to conserve the separate identities of the original terminal methyls.

It therefore appears that the basic stereochemical constraints in isoprenoid biosynthesis are the same in archaebacteria as in eubacteria and eukaryotes.

Glycerol Ether Formation

The glycerol ether formation is one of the most important biochemical steps common to the biosynthesis of all archaebacterial lipids. The formation of the glycerol diether was first investigated by *Kates* et al. (1970) in *Halobacterium · cutirubrum*, an obligate halophilic archaebacterium. In the incorporation experiments, using differently labelled glycerols as precursors, they found that the label from [U–^{14}C, 1(3)–^{3}H] glycerol was incorporated intact into the glycerol moiety of the diether lipids of *H. cutirubrum*, with retention of the initial $^{3}H/^{14}C$ ratio. In contrast, the label from [U–^{14}C, 2–^{3}H] glycerol was incorporated with an almost complete loss of ^{3}H. These authors regarded the loss of tritium, from the β-carbon of glycerol during the glycerol diether formation as a significant feature of the etherification step. They suggested that a more likely precursor would be dihydroxyacetone, which could be formed by the action of glycerol dehydrogenase, an enzyme known to be active in *H. cutirubrum* (*Kates* et al., 1970; *Kates*, 1972; *Kates* et al., 1978). Moreover, they excluded the participation of aldo-keto isomerization, such as that occurring between dihydroxyacetone phosphate and glyceraldehyde-3-phosphate, in the glycerol utilization for the glycerol moiety of diether lipids.

Different results were obtained with similar experiments in *Sulfolobus solfataricus* (*De Rosa* et al., 1982 b). To obtain an unequivocal rationalization of incoporation data with double-labelled glycerols in *S. solfataricus*, the possible role of isotopic effects due to the discriminatory capability against ^{3}H of enzymes involved in glycerol utilization has been preliminarily evaluated. Such an effect has been observed in *Mycoplasma* and rat liver by *Plackett* and *Rodwell* (1970) and *Manning* and *Brindley* (1972). These authors reported a significant isotopic effect of *sn*-glycero-3-phosphate dehydrogenase (EC 1.1.99.5), which discriminates against *sn*-[2–^{3}H]-glycerol-3-phosphate during oxidation. This effect, causing an increase of the $^{3}H/^{14}C$ ratio in the *sn*-glycerol-3-phosphate pool, represents a limit in the correct evaluation of incorporation data. In *S. solfataricus*, on the contrary, the enzymes directly involved in the glycerol utilization are not able to discriminate against ^{3}H. In fact, the isotope ratio of the glycerol-glycerol phosphate pool in the microorganism upon application of both [U–^{14}C, 2–^{3}H]glycerol and [U–^{14}C,1(3)–^{3}H]glycerol as precursors, does not change with respect to the original values of the fed precursors.

Table 1 summarizes the distribution of radioactivity in the macrocyclic tetraethers GDGT for the experiments employing the two differently labelled precursors (Fig. 3 a–i).

Table 1. Distribution of radioactivity in glycerol-dialkyl-glycerol-tetraethers (GDGT) of *Sulfolobus sulfataricus*

Compounds	Precursors $^{3}H/^{14}C$ = 3 [U–^{14}C, 1 (3)–^{3}H]glycerol Radioactivity (10^{-3} × dpm/mmol) ^{14}C	^{3}H	$^{3}H/^{14}C$	[U–^{14}C, 2–^{3}H]glycerol ^{14}C	^{3}H	$^{3}H/^{14}C$
GDGT	5880	15400	2.62	6000	14600	2.43
C_{40}Dichlorides from GDGT	590	830	1.41	631	90	0.14
Glycerol from GDGT	2310	6930	3.00	2348	7000	2.98

GDGT were obtained from complex lipids of *S. solfataricus* by methanolic HCl hydrolysis. The degradation products C_{40}dichlorides and glycerol were obtained by BCl_3 cleavage (*De Rosa* et al., 1982).

The $^3H/^{14}C$ ratio of GDGT in the two experiments decreases only slightly; this decrease was more evident with [U–^{14}C, 2–^{3}H] glycerol. The analysis of radioactivity distribution in the tetraethers, based on BCl_3 cleavage (*De Rosa* et al., 1982 b), shows that the glycerol moieties of the GDGT, using both double-labelled precursors, incorporate the radioactivity without any change in the $^3H/^{14}C$ ratio and with a high efficiency. In fact, the dilution of both glycerol precursors in the glycerol moieties is only 181-fold.

Conversely, the $^3H/^{14}C$ ratio in the C_{40} isoprenoids depends strictly on the ^{3}H position in the precursor; whereas [U–^{14}C, 1(3)–^{3}H] glycerol labels the isoprenoids with 50% of ^{3}H recovery, ^{3}H is absent in the [U–^{14}C, 2–^{3}H] glycerol incorporation experiment.

These results are further evidence of a classic mevalonate pathway in archaebacteria, as previously demonstrated by incorporation studies of more direct precursors such as acetate and mevalonate (*De Rosa* et al., 1977 b; *Langworthy* et al., 1974).

In relation to the mechanism of the glycerol ether assembly in *S. solfataricus*, the results on labelling of glycerol moieties of GDGT show that the ether forming step occurs without any loss of hydrogen from any of the glycerol carbons and therefore without the intervening formation of any oxidized intermediate derivative of the glycerol. Given the well demonstrated ability of geranyl-geranyl and similar allylic pyrophosphates to act as alkylating agents in other biosynthetic mechanisms, direct ether formation from glycerol (or, facilitated by neighbouring group deprotonation, from glycerolphosphate) presents no conceptual difficulties.

On the other hand, such alkylating reactivity would be lessened in a non-allylic (phytanyl) pyrophosphate, and this could be considered as evidence that ether formation precedes a reduction in the isoprenoid part of the ether lipids in *S. solfataricus*. According to this hypothesis, the unusual configuration of the chiral centre in the glycerol moiety would depend, in this microorganism, on the stereospecific nature of the alkylation step.

The proposed mechanism of glycerol ether formation in *S. solfataricus*, as depicted in Fig. 5, differs from that suggested by *Kates* (1972), who obtained both direct and presumptive evidence for a general pathway of glycerol metabolism in halobacteria. Although their conclusions are fully consistent with their observations, they throw no light on the mechanisms of ether lipid formation. In fact, these authors regard the loss of ^{3}H from the β-carbon of glycerol during the course of glycerol diether formation as a significant feature of the etherification step, whereas results obtained with *S. solfataricus* favour the alternative explanation that in halobacteria ^{3}H loss from the β-carbon is probably due to an efficient interconversion of glycerol (and/or glycero-phosphate and dihydroxyacetone and/or phosphate).

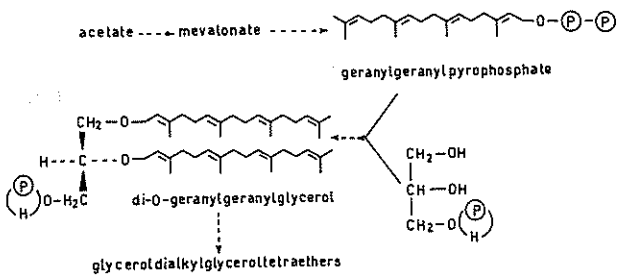

Fig. 5. Hypothesized mechanism of glycerol ether formation in *Sulfolobus solfataricus*.

Biogenesis of Calditol, the Branched Chain Nonitol Occurring in Tetraethers (GDNT) of *Sulfolobus* Species

Calditol is the unique branched-chain nonitol present in the GDNT tetraethers (Fig. 3 a′–i′), basic components of most of the complex membrane lipids of the *Sulfolobus* genus (*Langworthy* et al., 1974; *De Rosa* et al., 1980 b; *De Rosa* et al., 1980 d; *De Rosa* et al., 1983 b).

The studies of the biogenetic origin and assembly of the carbon skeleton of the calditol are based both on hydrolysis of ether linkage to recover structural elements of the tetraethers and on selective periodate degradation of the calditol moiety to establish the labelling pattern of the polyol with different labelled precursors (*De Rosa* et al., 1980 d).

Fig. 6 shows the labelling pattern in the calditol moiety using different double labelled precursors. With [U–^{14}C, 1(3)–^{3}H] and [U–^{14}C, 2–^{3}H] glycerols the radioactivity was localized specifically and with high efficiency on the carbons 1 to 3 of the calditol skeleton. In fact, about 98% of the calditol radioactivity was recovered on carbons 1 to 3 of the polyol with a dilution value (^{14}C specific activity of precursor/^{14}C specific activity of product) of about 60.

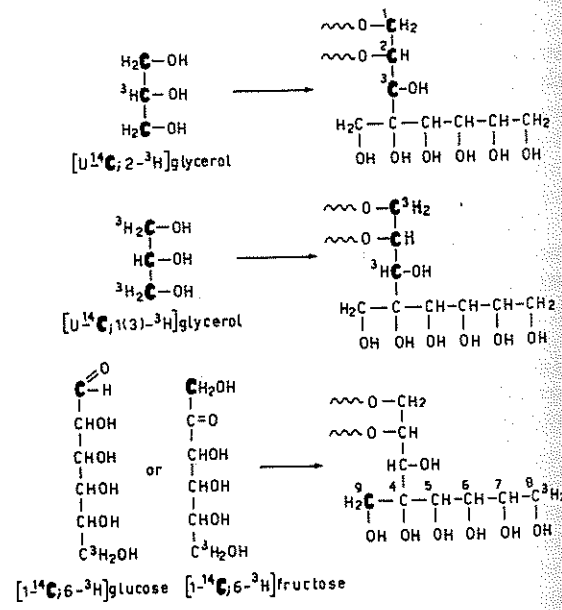

Fig. 6. Labelling pattern in the calditol moiety of GDNT using [U–^{14}C, 1(3)–^{3}H] and [U–^{14}C, 2–^{3}H glycerols], D-[1–^{14}C, 6–^{3}H glucose] and D-[1–^{14}C, 6–^{3}H] fructose as precursors.

Considering that the glycerol ether formation occurs without any loss of the ^{3}H from the precursor, as described above, the total loss of the ^{3}H from the β-carbon, of the glycerol precursor is evidence that a C-3 carbonilic intermediate, such as dihydroxyacetone or its activated derivative, is involved in the calditol skeleton assembly. Moreover, this result also indicates that the calditol skeleton biosynthesis occurs before the tetraether assembly.

Incorporation experiments with double labelled D-[1–^{14}C, 6-^{3}H] glucose and D-[1–^{14}C, 6–^{3}H] fructose show that both hexoses act as specific precursors of carbons 4 to 9 of the calditol skeleton (Fig. 6). About 90% of the calditol radioactivity is specifically localized on carbons 4 to 9 of the polyol skeleton, with retention of the original $^{3}H/^{14}C$ ratio and with a dilution value of about 175. Degradative experiments performed on labelled GDNT permit the localization of ^{14}C at carbon 9 and ^{3}H at carbon 8 of the polyol skeleton. Other hexoses, such as galactose, are not incorporated into the calditol moiety, thus indicating a critical role of the chiral centers of the sugar precursors.

The metabolic equivalence of glucose and fructose in the calditol biosynthesis is a consequence of an active interconversion of these hexoses via their 6-phosphate derivatives. Kinetic and chemicophysical properties of the enzymes involved in this pattern have been studied (*De Rosa* et al., 1984).

The complex of evidence provided by the labelling pattern of calditol from experiments employing differently labelled precursors lead us to conclude that – without regarding implications as to stereochemistry or phosphorylation – the biosynthesis of calditol occurs via an aldolic condensation between dihydroxyacetone and fructose, giving rise to, as an intermediate, a 2-cheto-derivative of calditol. The latter is in turn reduced and then alkylated to yield the glycerol-dialkyl-calditol tetraethers (GDNT) (pers. comm. of the authors).

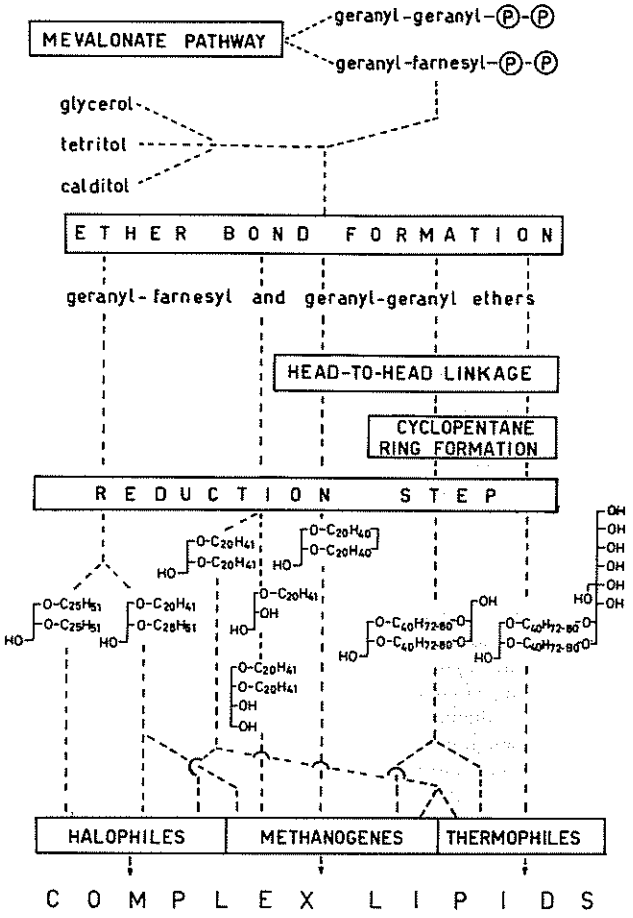

Fig. 7. Pathway of ether lipid assembly in archaebacteria.

Final Remarks

Biogenetic studies and structural constraints of isoprenoid ether lipids enable us to propose a hypothetical sequence for the assembly of these molecules that appears more plausible and economical than do alternative pathways. Without considering implications as to the activation of the intermediate, this scheme is set out in Fig. 7.

Ether bond formation between low molecular weight alcohols (glycerol, tetritol or calditol) and geranyl-gezanyl-pyrosphosphate or geranyl-farnesyl-pyrophosphate is the common biosynthetic step in archaebacteria lipid biogenesis. In halophiles hydrogenation of unsaturated intermediates leads to the formation of glycerol diether lipids, while in methanogens and thermophiles, in competition with the reduction step, it effects head-to-head coupling between C_{20} unsaturated isoprenoid chains and cyclopentane ring formation to give rise to a variety of structural types of lipids characteristic of these phenotypes.

References

Balch, W. E., Magrum, L. J., Fox, G. E., Wolfe, R. S., Woese, C. R.: An ancient divergence among the bacteria. J. Molec. Evol. *9*, 305–311 (1977)

Bu'Lock, J. D., De Rosa, M., Gambacorta, A.: Isoprenoid Biosynthesis in Archaebacteria. In: Biosynthesis of isoprenoid compounds (*J. W. Porter* and *S. L. Spurgeon*, eds.), 2nd, p. 159–189. New York, John Wiley and Sons 1983

Comita, P. B., Gagosian, R. B.: Membrane lipid from deep sea hydrothermal vent methanogen: a new macrocyclic glycerol diether. Science *222*, 1329–1331 (1983)

Comita, P. B., Gagosian, R. B., Pang, H., Costello, C. E.: Structural elucidation of a unique macrocyclic membrane lipid from a new, extremely thermophilic, deep-sea hydrothermal vent archaebacterium *Methanococcus jannaschii.* J. Biol. Chem. *259*, 15234–15241 (1984)

De Rosa, M., Gambacorta, A., Bu'Lock, J. D.: Extremely thermophilic acidophilic bacteria convergent with *Sulfolobus acidocaldarius.* J. gen. Microbiol. *86*, 156–164 (1975)

De Rosa, M., De Rosa, S., Gambacorta, A., Minale, L., Bu'Lock, J. D.: Chemical structure of the ether lipids of thermophilic

acidophilic bacteria of the *Caldariella* group. Phytochemistry *16*, 1961–1965 (1977 a)

De Rosa, M., De Rosa, S., Gambacorta, A.: ^{13}C-NMR assignment and biosynthetic data for the ether lipids of *Caldariella*. Phytochemistry *16*, 1909–1912 (1977 b)

De Rosa, M., Esposito, E., Gambacorta, A., Nicolaus, B., Bu'Lock, J. D.: Effects of temperature on ether lipid composition of *Caldariella acidophila*. Phytochemistry *19*, 827–831 (1980 a)

De Rosa, M., Gambacorta, A., Nicolaus, B., Bu'Lock, J. D.: Complex lipids of *Caldariella acidophila*, a thermoacidophile archaebacterium. Phytochemistry *19*, 822–825 (1980 b)

De Rosa, M., Gambacorta, A., Nicolaus, B.: Regularity of isoprenoid biosynthesis in the ether lipids of archaebacteria. Phytochemistry *19*, 791–793 (1980 c)

De Rosa, M., De Rosa, S., Gambacorta, A., Bu'Lock, J. D.: Structure of calditol, a new branched-chain nonitol, and of the derived tetraether lipids in thermoacidophile archaebacteria of the *Caldariella* group. Phytochemistry *19*, 249–254 (1980 d)

De Rosa, M., Gambacorta, A., Nicolaus, B., Ross, H. N. M., Grant, W. D., Bu'Lock, J. D.: An asymmetric archaebacterial diether lipid from alkaliphilic halophiles. J. gen. Microbiol. *128*, 343–348 (1982 a)

De Rosa, M., Gambacorta, A., Nicolaus, B., Sodano, S.: Incorporation of labelled glycerols into ether lipids in *Caldariella acidophila*. Phytochemistry *21*, 595–599 (1982 b)

De Rosa, M., Gambacorta, A., Nicolaus, B., Grant, W. D.: A C_{25}, C_{25} diether core lipid from archaebacterial haloalkaliphiles. J. gen. Microbiol. *129*, 2333–2337 (1983 a)

De Rosa, M., Gambacorta, A., Nicolaus, B., Chappe, B., Albrecht, P.: Isoprenoid ethers; backbone of complex lipids of the archaebacterium *Sulfolobus solfataricus*. Biochim. Biophys. Acta *53*, 249–256 (1983 b)

De Rosa, M., Gambacorta, A., Nicolaus, B., Giardina, P., Poerio, E., Buonocore, V.: Glucose metabolism in the extreme thermoacidophilic archaebacterium *Sulfolobus solfataricus*. Biochem. J. *224*, 407–414 (1984)

De Rosa, M., Gambacorta, A., Gliozzi, A.: Structure, biosynthesis and physico-chemical properties of archaebacteria lipids. Microbiol. Rev. *50*, in press (1986 a)

De Rosa, M., Gambacorta, A., Lanzotti, V., Trincone, A., Harris, J. E., Grant, W. D.: A range of ether core lipids from the methanogenic archaebacterium *Methanosarcina barkeri*. Biochim. Biophys. Acta, in press (1986 b)

De Rosa, M., Gambacorta, A., Trincone, A., Basso, A., Zillig, W., Holz, I.: Lipids of *Thermococcus celer*, a sulfur-reducing archaebacterium: structure and biosynthesis. System. Appl. Microbiol. *8*, in press (1986 c)

Gaber, S. H., Truper, H. G.: Halobacterium pharaonis with low magnesium requirement. In: Archaebacteria (*O. Kandler*, ed.), p. 318–329. Stuttgart, Gustav Fischer Verlag 1982

Grant, W. D., Pinch, G., Harris, J. E., De Rosa, M., Gambacorta, A.: Polar lipids in methanogen taxonomy. J. gen. Microbiol. *131*, 3277–3286 (1985)

Javor, B., Requadt, C., Stoeckenius, W.: Box-shaped halophilic bacteria. J. Bact. *151*, 1532–1542 (1982)

Kates, M., Wassel, M. K., Pugh, E. C.: Origin of the glycerol moieties in the glycerol diether lipids of *Halobacterium cutirubrum*. Biochim. Biophys. Acta *202*, 206–208 (1970)

Kates, M.: Ether-linked lipids in extremely halophilic bacteria. In: Ether lipids, Chemistry and Biology (*F. Snyder*, ed.), p. 351–398. New York, Academic Press 1972

Kates, M., Kushwaha, S. C.: Biochemistry of the lipids of extremely halophilic bacteria. In: Energetics and Structure of Halophilic Microorganisms (*S. R. Caplan* and *M. Ginzburg*, eds.), p. 461–479. Amsterdam, Elsevier, North Holland Biomedical Press 1978

Kushwaha, S. C., Kates, M., Sprott, G. D., Smith, I. C. P.: Novel complex polar lipids from the methanogenic archaebacterium *Methanospirillum hungatei*. Science *211*, 1163–1164 (1981 a)

Kushwaha, S. C., Kates, M., Sprott, G. D., Smith, I. C. P.: Novel polar lipids from the methanogen *Methanospirillum hungatei* GP1. Biochim. Biophys. Acta *664*, 156–173 (1981 b)

Kushwaha, S. C., Kates, M., Juez, G., Rodriguez-Valera, F., Kushner, D. J.: Polar lipids of an extremely halophilic bacterial strain (R–4) isolated from salt ponds in Spain. Biochim. Biophys. Acta *711*, 19–25 (1982)

Langworthy, T. A., Smith, P. F., Mayberry, W. R.: Lipids of *Thermoplasma acidophilum*. J. Bact. *112*, 1193–1200 (1972)

Langworthy, T. A., Mayberry, W. R., Smith, P. F.: Long chain diether and polyol dialkyl glycerol triether lipids of *Sulfolobus acidocaldarius*. J. Bact. *119*, 106–116 (1974)

Langworthy, T. A.: Comparative lipid composition of heterotrophically and autotrophically grown *Sulfolobus acidocaldarius*. J. Bact. *130*, 1326–1332 (1977)

Langworthy, T. A.: Special features of thermoplasmas. In: The Mycoplasmas, Vol. 1 (*M. F. Barile* and *S. Razin*, eds.), p. 495–513. New York, Academic Press 1979

Langworthy, T. A.: Tornabene, T. G., Holzer, G.: Lipids of archaebacteria. In: Archaebacteria (*O. Kandler*, ed.), p. 228–244. Stuttgart, Gustav Fischer Verlag 1982

Makula, R. A., Singer, M. E.: Ether containing lipids of methanogenic bacteria. Biochim. Biophys. Res. Commun. *82*, 716–722 (1978)

Manning, R., Brindley, D. M.: Tritium isotope effects in the measurements of the glycerol phosphate and dihydroxyacetone phosphate pathways of glycerolipid biosynthesis in rat liver. Biochem. J. *130*, 1003–1012 (1972)

Plackett, P., Rodwell, A. W.: Glycerolipid biosynthesis by Mycoplasma strain y. Biochim. Biophys. Acta *210*, 230–240 (1970)

Ross, H. N. M., Grant, W. D.: Nucleic acid studies on halophilic archaebacteria. J. gen. Microbiol. *131*, 165–173 (1985)

Stetter, K. O., König, H., Stackebrandt, E.: Pyrodictium gen. nov., a new genus of submarine disc-shaped sulphur reducing archaebacteria growing optimally at 105 °C. System. Appl. Microbiol. *4*, 535–551 (1983)

Tindall, B. J., Ross, H. N. M., Grant, W. D.: Natronobacterium gen. nov. and *Natronococcus* gen. nov., two new genera of haloalkaliphilic archaebacteria. System. Appl. Microbiol. *5*, 41–57 (1984)

Woese, C. R., Fox, G. E.: Phylogenetic structure of the prokaryotic domain: The primary kingdoms. Proc. nat. Acad. Sci. (Wash.) *74*, 5088–5090 (1977)

Woese, C. R.: Archaebacteria and cellular origins: an overview. In: Archaebacteria (*O. Kandler*, ed.), p. 1–17. Stuttgart, Gustav Fischer Verlag 1982

Zeikus, J. G.: The biology of methanogenic bacteria. Bact. Rev. *41*, 514–541 (1977)

Zeikus, J. G., Aric Ben-Bassat, Heggen, P. W.: Microbiology of methanogensis in thermic volcanic environments. J. Bact. *143*, 432–440 (1980)

Zillig, W., Stetter, K. O., Wunderl, S., Schulz, W., Priess, H., Scholz, I.: The *Sulfolobus "Caldariella"* group: taxonomy on the basis of the structure of DNA-dependent RNA polymerases. Arch. Microbiol. *125*, 259–269 (1980)

Zillig, W., Stetter, K. O., Schafer, W., Janekovic, D., Wunderl, S., Holz, I., Palm, P.: Thermoproteales: a novel type of extremely thermoacidophilic anaerobic archaebacteria isolated from Icelandic solfataras. Zbl. Bakt. Hyg., I. Abt. Orig. C *2*, 205–227 (1981)

Zillig, W., Stetter, K. O., Prangishvilli, D., Schafer, W., Wunderl, S., Janekovic, D., Holz, I., Palm., P.: Desulfurococcaceae, the second family of the extremely thermophilic, anaerobic, sulphur-respiring *Thermoproteales.* In: Archaebacteria (*O. Kandler,* ed.), p. 304–317. Stuttgart, Gustav Fischer Verlag 1982

Zillig, W., Gierl, A., Schreiber, G., Wunderl, S., Janekovic, D., Stetter, K. O., Klenk, H. P.: The archaebacterium *Thermofilum pendens* represents a novel genus of thermophilic anaerobic sulphur-respiring *Thermoproteales.* System. Appl. Microbiol. *4,* 79–87 (1983)

Dr. *Mario De Rosa,* Istituto per la Chimica di Molecole di Interesse Biologico, C.N.R., Via Toiano n. 6, I-80072 Arco Felice, Napoli, Italy