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Structural complexity in isoprenoid glycerol dialkyl glycerol tetraether lipid cores of *Sulfolobus* and other archaea revealed by liquid chromatography–tandem mass spectrometry

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ABSTRACT

Liquid chromatography–tandem mass spectrometry of membrane lipid cores from *Sulfolobus* species reveals isomeric forms of ring-containing isoprenoid glycerol dialkyl glycerol tetraether components not previously recognised via the use of NMR and liquid chromatography–mass spectrometry techniques. Equivalent isomerism was confirmed for the components in other hyperthermophilic genera and in sediments which contain the lipids of mesophilic archaea. The recognition of the isomeric structures in distinct archaeal clades suggests that profiles of tetraether lipids reported previously may have oversimplified the true lipid complexity in archaeal cultures and natural environments. Accordingly, the extent of variation in tetraether structures revealed by the work should direct more informative interpretations of lipid profiles in the future. Moreover, the results emphasise that tandem mass spectrometry provides a unique capability for assigning the structures of intact tetraether lipid cores for co-eluting species during chromatographic separation.

1. Introduction

Isoprenoid tetraether lipids are characteristic components of the membranes of some Archaea (Woese, 1987), including both extremophilic (De Rosa and Gambacorta, 1988; Schouten et al., 2007) and mesophilic (Wuchter et al., 2004; Pitcher et al., 2011) species. The complex archaeal lipids consist of an ether lipid core appended with glycosyl and/or modified phosphate polar head groups (Sturt et al., 2004), moieties which are hydrolysed following cell senescence in natural environments (Harvey et al., 1986; Schouten et al., 2010) or which can be removed via artificial hydrolysis or methanolysis (Schouten et al., 2008a; Knappy et al., 2009). A limited variety of archaeal tetraether lipid cores were revealed during classic studies (De Rosa et al., 1977a, b, c, 1980a, b, 1983; De Rosa and Gambacorta, 1988) of the MT-3 and MT-4 strains of the hyperthermophilic Crenarchaeon *Sulfolobus solfataricus*.[1] Degradation of complex lipids extracted from the organism by methanolysis and subsequent treatment with HI-$LiAlH_4$ or BCl_3-$LiAlH_4$ led to the reductive cleavage of ether linkages and the liberation of biphytane (**a**) and related hydrocarbons (**b**–**e**) containing 1-4 cyclopentyl rings (De Rosa et al., 1977a, b, c, 1980a). These chains were shown to originate from two distinct structural types (De Rosa et al., 1977a, c, 1980a); from glycerol dialkyl glycerol tetraether (GDGT) lipid cores, in which the biphytanes are etherified to two glycerol termini, or from glycerol dialkyl calditol tetraether (GDCT) cores, in which they are etherified to one glycerol and one calditol group (Fig. 1).[2] Isolation of individual lipid components by preparative chromatography followed by

Abbreviations: GDGT, glycerol dialkyl glycerol tetraether; GDCT, glycerol dialkyl calditol tetraether; LC–MS, liquid chromatography–mass spectrometry; LC–MS/MS, liquid chromatography–tandem mass spectrometry; CID, collision induced dissociation; GC, gas chromatography; GC–MS, gas chromatography–mass spectrometry; NZ, New Zealand; DCM, dichloromethane; Da, daltons; Ma, million years.

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[1] *S. solfataricus* is a recent phyletic reclassification of "*Caldariella acidophila*", the nomenclature used for the organism prior to 1980 (Zillig et al., 1980).

[2] The original structure proposed for calditol (i.e. as an open chained nonitol; De Rosa et al., 1980a) was corrected more recently (Blériot et al., 2002) to the moiety shown in Fig. 1.

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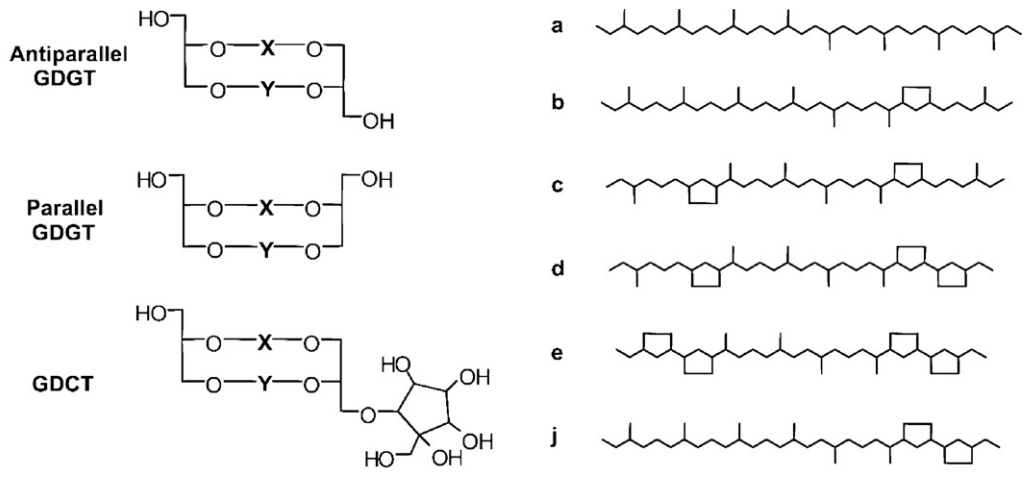

GDGT structures				Occurrences[b]		
Structure No.	Designation[a] (**X** , **Y**)	Isomeric[b] form(s)	Designation (**X** , **Y**)	*S. acid.* MR31	*S. shib.* B12	*S. solf.* P2
0	Δ_0 (**a** , **a**)			+	+	(+)
1	Δ_1 (**a** , **b**)			+	+	(+)
		1'	?	(+)	-	-
2	Δ_0 (**b** , **b**)	**2**	Δ_2 (**a** , **c**)	+	+	(+)
		2'	?	(+)	-	-
3	Δ_1 (**b** , **c**)			+	+	+
		3'	Δ_1 (**b** , **c**)	+	+	+
4	Δ_0 (**c** , **c**)			+	+	+
		4'	Δ_0 (**c** , **c**)	+	+	+
		4''	Δ_0 (**c** , **c**)	+	+	+
		4''	Δ_2 (**b** , **d**)	+	+	+
5	Δ_1 (**c** , **d**)			+	+	+
		5'	Δ_1 (**c** , **d**)	+	+	+
6	Δ_0 (**d** , **d**)	**6**	Δ_2 (**c** , **e**)	+	+	+
7	Δ_1 (**d** , **e**)			(+)	+	+
8	Δ_0 (**e** , **e**)			-	+	+

Fig. 1. Structures discussed in the text, with the isomeric form (i.e. Δ_0, Δ_1 or Δ_2) and the chain designations provided for the GDGT structures. The occurrences of the lipids in three strains of *Sulfolobus* are also provided. Superscript letters a and b denote structures reported by De Rosa and Gambacorta (1988) and additional isomers revealed during the present study, respectively. +, identified in the organism via MS and MS/MS spectral data; (+), identified in the organism via MS spectral data alone (i.e. an MS/MS spectrum could not be obtained); –, not detected in the organism.

reduction to the biphytanyl chains allowed identification of components containing up to eight cyclopentyl rings (e.g. GDGTs **0**–**8**; Fig. 1) in each structural class (De Rosa et al., 1980b, 1983). The lipid cores containing an even number of cyclopentyl rings were attributed structures in which the two etherified chains are identical (i.e. Δ_0 structures; De Rosa et al., 1980b), whereas those containing an odd number of rings were ascribed inequivalent isoprenoid chains containing numbers of rings which differ by one (i.e. Δ_1 structures; De Rosa et al., 1983). The *sn*-2 and *sn*-3 carbinols of the glycerol or calditol groups were confirmed as the sites of etherification, with the two glycerol groups in GDGT lipids originally suggested to be aligned antiparallel to one-another (De Rosa et al., 1980b, 1983). More recently, however, the lipids in *S. solfataricus* P1 (Gräther and Arigoni, 1995) have been shown to exist as a near equimolar mixture of antiparallel and parallel isomeric forms (Fig. 1).