Note

Structural Diversity of the Membrane Core Lipids of Extreme Halophiles

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Received October 3, 1997

The structural diversity of the core lipids of extreme halophiles *Haloarcula japonica* and *Halobacterium halobium* was investigated. The most significant difference is that *Ha. japonica* contains *sn*-2,3-di-*O*-phytanylglycerol exclusively as the core lipid, whereas *Hb. halobium* contains both *sn*-2,3-di-*O*-phytanylglycerol and *sn*-2-*O*-sesterterpanyl (3,7,11,15,19-pentamethyleicosanyl)-3-*O*-phytanylglycerol.

Key words: archaea; halophile; membrane lipid

Archaea (archaeobacteria), including extreme thermophiles, extreme halophiles, extreme acidophiles, and methanogen, are known to contain a dipolyprenylated glycerol structure as their membrane core lipid.¹⁾ Horikoshi *et al.* have reported the unique cell shape of *Haloarcula japonica* grown under certain conditions,²⁾ which prompted us to look more closely into the composition and structure of membrane core lipids as part of our continuing chemical studies of archaeal membrane lipids.

Ha. japonica JCM7785 was obtained from RIKEN and cultivated at 37°C for 6–7 days in a medium containing yeast extract (10 g), casamino acid (7.5 g), NaCl (200 g), MgSO₄·7H₂O (20 g), trisodium citrate·2H₂O (3 g), KCl (2 g), and FeSO₄·7H₂O (50 mg) in 1 litre of water (pH 7.4). *Halobacterium halobium* IAM13167 cells, which had been prepared by growing under the conditions previously reported,³⁾ were used as the reference. Total polar lipids were extracted from the wet cells with CHCl₃-MeOH-water according to the procedure of Minnikin *et al.*,⁴⁾ and a TLC analysis of the total non-hydrolyzed lipid fraction of *Ha. japonica* clearly verified the existence of the unknown lipid spots that were expected according to the report by Horikoshi *et al.*²⁾ What was interesting here was that duplicated or overlapping spots were observed on the TLC plate of the *Hb. halobium* lipid. While the lipid of *Hb. halobium* is known to basically contain 2,3-di-*O*-phytanylglycerol (**1a**) as the core structure,¹⁾ the present observations suggest the co-existence of another homologue. Dried cells of both species were separately heated in H₂SO₄-MeOH-toluene at 50°C for 15 h to yield the core lipid fractions,⁵⁾ which were further purified by absorption column chromatography on silica gel to afford core lipid specimens. The electron-ionization mass (EI-MS) spectral analysis of the hydrolyzed lipid from *Hb. halobium* clearly suggested the existence of C₂₀,C₂₅-glycerol **2a** (M⁺ *m/z* 722) in addition to well established 2,3-di-*O*-phytanylglycerol (M⁺ *m/z* 652) in an approximate

ratio of 1:2. The hydrolyzed specimen of *Ha. japonica* appeared to comprise exclusively 2,3-di-*O*-phytanylglycerol (**1a**) as its core lipid. We have not examined the possibility of the C₄₀,C₄₀-diglycerol tetraether¹⁾ at this time.

De Rosa *et al.* have previously shown that *sn*-2-*O*-sesterterpanyl-3-*O*-phytanylglycerol lipids exist in alkalophilic halophiles.⁶⁾ The existence of a similar C₂₀,C₂₅-glycerol lipid in *Hb. halobium*, which is a non-alkalophilic halophile, has also been suggested by others without any physicochemical evidence.^{5,7)} We thus pursued further purification and isolation of C₂₀,C₂₅-glycerol lipid **2a** for an unequivocal physical characterization. As anticipated, separation of C₂₀,C₂₅-glycerol **2a** and 2,3-di-*O*-phytanylglycerol **1a** was most tedious, but was carefully carried out. Benzoylation of the lipids was carried out in the conventional way (benzoyl chloride/pyridine, 0°C), and the resulting benzoates were separated by centrifugal partition chromatography (CPC) with hexane-methanol as the solvent system to yield chromatographically homogeneous specimens of benzoylated 2,3-di-*O*-phytanylglycerol **1b** and C₂₀,C₂₅-glycerol **2b**.

The purified benzoates were subsequently subjected to EI-MS and NMR analyses. The EI-MS data of **1b** and **2b** clearly showed the molecular ion peaks at *m/z* 756 and 826, respectively. The most crucial was the ion formed by fission between the C-2 and C-3 bond of the glycerol portion.⁶⁾ The ion of *m/z* 515 that was observed as a base peak of the spectrum of **2b** allowed us to unambiguously assign the location (C-2 of glycerol) of the sesterterpanyl hydrophobic chain as shown in Fig. 2. An ion of *m/z* 445 would have been observed if the lipid had contained the phytanyl (C₂₀) chain at the C-2 position of the glycerol. It now appears that the glycerolipid of *Hb. halobium* contains lipids having a sesterterpanyl chain at the C-2 position of glycerol. As so far examined, 2,3-di-*O*-sesterterpanylglycerol⁸⁾ or other higher homologues have not been detected on the mass spec-

Fig. 1.

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Fig. 2. Electron-ionization Mass Spectra of Benzoylated Core Membrane Lipids **1b** and **2b** from Extreme Halophiles *Ha. japonica* and *Hb. halobium*.

trum. The ^1H - and ^{13}C -NMR spectra of **1b** were completely identical with those of an authentic synthetic sample.^{3,9)} The ^1H - and ^{13}C -NMR spectra of **2b** were quite similar to those of **1b**. Although the absolute stereochemistry of **2b** has not been rigorously determined in this study, it appears most likely that the hydrophobic alkyl chains are linked to the *sn*-2- and 3-positions of glycerol, because all of the archaeal lipids reported to date have the same configuration.¹⁾ Furthermore, since the chemical metabolism in microorganisms is usually well programmed, the stereochemistry of the sesterterpanyl part of **2a** in *Hb. halobium* is also likely to be similar to the related phytanyl structures.

In conclusion, the core lipids of extreme halophiles

are quite different according to species. *Ha. japonica* contains exclusively *sn*-2,3-di-*O*-phytanylglycerol (**1a**) as the core lipid, whereas *Hb. halobium* contains both of *sn*-2,3-di-*O*-phytanylglycerol (**1a**) and *sn*-2-*O*-sesterterpanyl-3-*O*-phytanylglycerol (**2a**).

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