The Stereochemical Fate of Glycerol during the Biosynthesis of Membrane Lipids in Thermoacidophilic Archaebacteria *Sulfolobus acidocaldarius*

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Chirally deuteriated glycerol has been incorporated stereospecifically into the membrane lipids, 2,3-di-*O*-biphytanyl diglycerol tetraether lipids, of acidothermophile *Sulfolobus acidocaldarius* in such a way that the sn-C-3 position of glycerol is alkylated, in contrast to the case of halophilic archaebacteria *Halobacterium halobium.*

A significant feature of the phospholipids and glycolipids of archaebacterial cell membranes is the stereochemistry of the glycerol in the core lipids. The glycerol is sn-2,3-di-*O*-alkylated but not sn-1,2-di-*O*-acylated as in eubacterial and eukaryotic cells.[1] Recently we reported that, in the biosynthesis of the membrane core lipid of halophilic archaebacteria *Halobacterium halobium*, the sn-C-3 hydroxymethyl group of glycerol and the C-6 hydroxymethyl group of D-glucose are stereospecifically converted to the sn-C-1 of the 2,3-di-*O*-phytanyl glycerol and stereochemical inversion at C-2 takes place through dehydrogenation to form dihydroxyacetone phosphate.[2,3] In contrast to this, De Rosa *et al.* reported earlier that the C-2 hydrogen of glycerol is retained in the membrane lipids of different archaebacteria *Sulfolobus sp.*[4] To clarify this difference, we have investigated further the biosynthetic mechanism of archaebacterial membrane lipids.

We followed deuteriated glycerol substrates during the formation of biphytanyl diglycerol tetraether lipids in *S. acidocaldarius* by ^2H NMR spectroscopy. The substrates, rac-[1,1-$^2H_2$]glycerol (**1**), sn-[3,3-$^2H_2$]glycerol (**2**), and [2-^2H]glycerol (**3**) were prepared as described previously.[2,3] These were then separately administered to a culture of *S. acidocaldarius*. The cells were grown at 80 °C for seven days in a medium (pH 3.5) composed of NaCl (0.2 g l^{-1}), KH_2PO_4 (3 g l^{-1}), $MgSO_4 \cdot 7H_2O$ (0.25 g l^{-1}), $CaCl_2 \cdot 2H_2O$ (0.05 g l^{-1}), $(NH_4)_2SO_4$ (1.3 g l^{-1}), D-glucose (1 g l^{-1}), yeast extract (1 g l^{-1}), and casamino acid (1 g l^{-1}). The lipids were extracted from the harvested cells according to the literature method.[4] Each deuterium-enriched lipid sample was purified by SiO_2

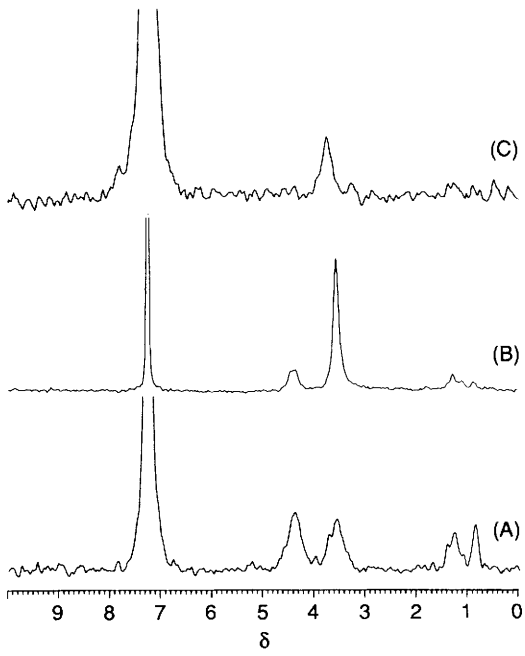
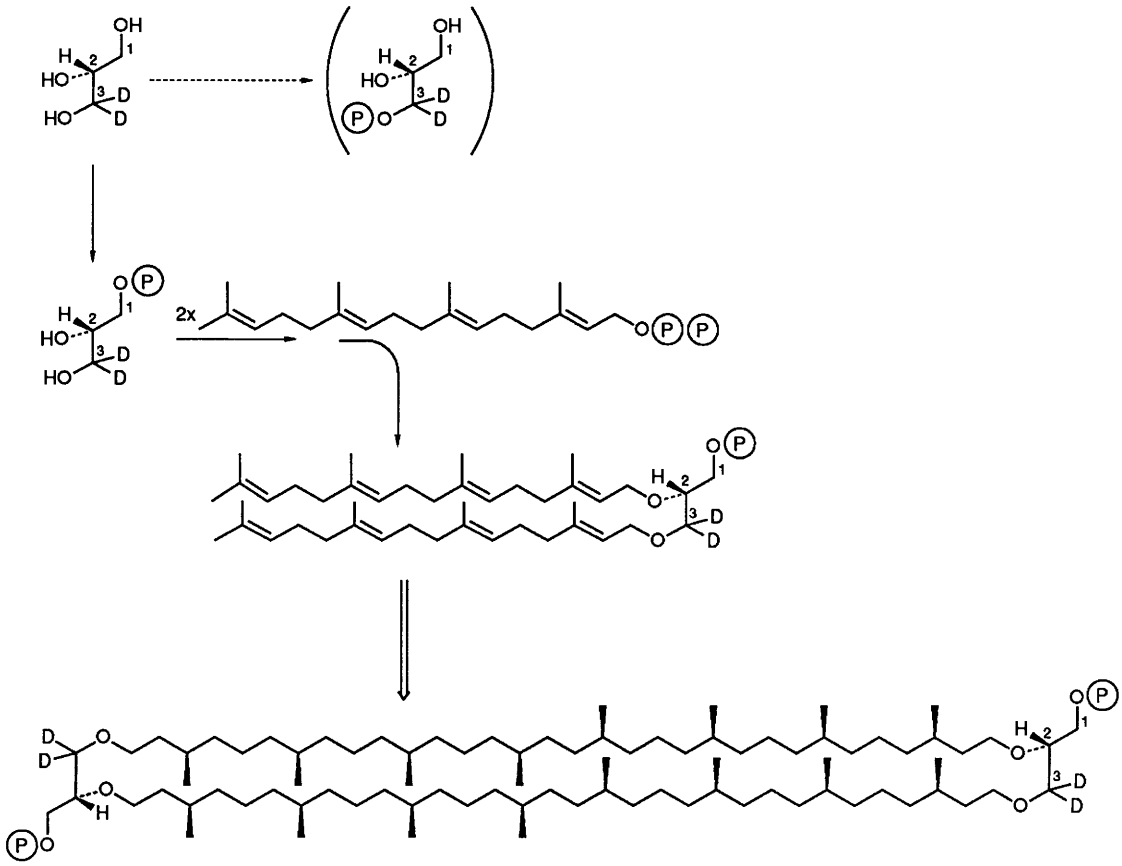


Figure 1. ^2H NMR spectra [76.8 MHz, $CHCl_3$, referenced to $CDCl_3$ (natural abundance) δ 7.26] of benzoylated lipid by feeding of (A) *rac*-[1,1-$^2H_2$]glycerol, (B) sn-[3,3-$^2H_2$]glycerol, and (C) [2-^2H]glycerol.



Scheme 1. Biosynthetic pathway of membrane lipid, biphytanyl diglycerol tetraether, in *S. acidocaldarius*.

column chromatography, converted to di-*O*-benzoate derivatives, by treatment with benzoyl chloride in pyridine, to differentiate the chemical shifts of the oxymethylene hydrogens, and further purified by preparative TLC. Although the structural microheterogeneity of the long chain alkyl portions of *S. acidocaldarius* lipids is known,[1] the NMR chemical shifts of the hydrogens in the glycerol moieties appear to be almost homogeneous even in the heterogeneous state. We therefore analysed the ^{2}H NMR spectrum of each lipid benzoate sample obtained from the feeding experiment with (**1**), (**2**) or (**3**) (Figure 1). The deuterium chemical shifts were assigned by comparison with well established ^{1}H NMR data of 1-*O*-benzoyl-2,3-di-*O*-phytanyl glycerol.[2,3] Although, unlike in *H. halobium*,[2,3] the deuterium enrichment was not high enough to allow estimation of the enrichment ratios using ^{1}H NMR or mass spectra, deuterium incorporation was clearly observed from the ^{2}H NMR spectra in each case.

The observation that the sn-C-1 position (δ_D 4.38) and sn-C-3 position (δ_D 3.55) were equally labelled with (**1**) [Figure 1(A)] implies direct incorporation of glycerol into the lipid as expected from previously reported results.[4] The terpene portions were also labelled to some extent, thereby suggesting viability of the acetate-mevalonate pathway in *S. acidocaldarius*.

To determine the metabolic difference between *H. halobium* and *Sulfolobus* species, we next investigated the cryptic stereochemistry of glycerol. We followed the deuterium atoms of (**2**), the chiral purity of which was determined to be 77% enantiomeric excess (e.e.) by a procedure described previously.[3] This chiral purity was helpful because the fate of each hydroxymethyl group of glycerol could be analysed in a single experiment. The ^{2}H NMR spectrum [Figure 1(B)] of the labelled lipid benzoate obtained from the feeding experiment with (**2**) showed signals at δ_D 4.38 and 3.55, the intensity ratio of which was in accord with the chiral purity of (**2**). This clearly implies that (**2**) was incorporated stereospecifically into the glycerol portion of the lipid in such a way that sn-C-3 of glycerol was *O*-prenylated and the sn-C-1 carbon remained attached to a polar head group, *i.e.*, the benzoylated site, in the lipids. This mode of glycerol incorporation is apparently opposite to that in *H. halobium*.[2,3]

The fate of the C-2 hydrogen of glycerol was re-examined as above. As can be seen in the Figure 1(C), the deuterium of (**3**) was confirmed to be incorporated into the glycerol moiety of the lipid by the signal at δ_D 3.75. This is consistent with the result reported by De Rosa *et al.*[4]

The most likely pathway which can account for overall retention of stereochemistry during di-*O*-alkylation of glycerol and retention of the C-2 hydrogen of glycerol appears to be one where glycerol is directly phosphorylated into sn-glycerol-1-phosphate in *S. acidocaldarius* rather than into sn-glycerol-3-phosphate, a plausible precursor in the lipid biosynthesis in *H. halobium*, as shown in the Scheme 1. The subsequent prenyl transfer reactions may afford a possible sn-2,3-di-*O*-prenylated glycerol intermediate.

Archaebacteria currently attract wide attention from evolutionary standpoints. The present results suggest that different fundamental metabolisms appear to operate depending upon the species of archaebacteria and more precise metabolic analyses are necessary for understanding in depth these intriguing bacterial species.

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† *Note added in proof:* Poulter *et al.* have recently reported that sn-glycerol-1-phosphate is the acceptor of geranylgeranyl groups in the prenyl transfer reactions catalysed by the cell-free extracts of methanogenic archaebacteria *Methanobacterium thermautotrophicum*. See D.-L. Zhang, L. Daniels, and C. D. Poulter, *J. Am. Chem. Soc.*, 1990, **112**, 1264.

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