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Unusual double-bond migration as a plausible key reaction in the biosynthesis of the isoprenoidal membrane lipids of methanogenic archaea†

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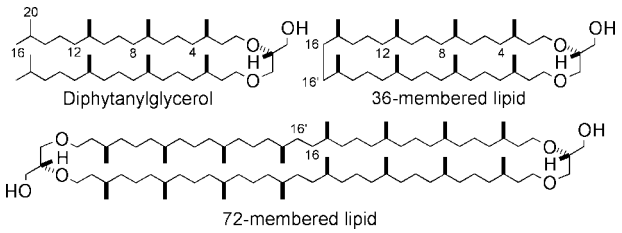
Labeling experiments of mevalonolactone-d_9 were pursued for studies of the biosynthesis of the core membrane lipids from thermophilic methanogenic archaea, which revealed that unusual double-bond migration occurred during the ultimate formation of the saturated isoprenoid chains in these particular archaea.

Archaea, which have been attracting considerable attention from both biochemical and evolutionary aspects, are distinct from bacteria and eukarya.[1] Archaeal cell membrane lipids are composed of isoprenoid-chain hydrocarbons linked to a glycerol molecule at the *sn*-2 and 3-positions by an ether linkage. The isoprenoid hydrocarbon chains of lipid molecules are frequently joined at the hydrophobic end to form a macrocyclic ring as large as 36- or 72-membered as shown in Scheme 1.[2]

The biosynthesis of these macrocyclic lipids is believed to proceed in a direct manner *via* digeranylgeranylglyceryl phosphate derived *sn*-glycerol 1-phosphate and geranylgeranyl diphosphate.[3] However, nothing is known about the mechanistic details of the final carbon–carbon bond(s) formation found in macrocyclic lipids. Described here are unanticipated observations from similar feeding experiments[4] of mevalonolactone-d_9 to thermophilic methanogens, *Methanococcus jannaschii* and *Methanobacterium thermoautotrophicum*.

The cultures of *M. jannaschii* (DSM 2661) and *M. thermoautotrophicum* JCM 10044^T (DSM 1053) were carried out as previously reported.[5,6] Mevalonolactone-d_9,[4] was aseptically and anaerobically added to the culture in a final concentration of 0.25 and 1.0 g L^{-1}, respectively. After cultivation the lipids were separately purified according to the standard procedure.[7] Repetitive chromatography afforded diphytanylglycerol, a 36-membered lipid, and a 72-membered lipid from *M. jannaschii*, and diphytanylglycerol and a 72-membered lipid from *M. thermoautotrophicum*, respectively. These core lipids were further converted to their corresponding benzoates and were first analyzed by ^{1}H NMR.

Fig. 1 shows pertinent ^{1}H NMR spectra of the benzoates of diphytanylglycerol, the 36-membered lipid, and the 72-membered lipid biosynthesized from mevalonate-d_9 from *M. jannaschii* and *M. thermoautotrophicum*. Also shown is the previously reported ^{1}H NMR spectra of perdeuterated diphytanylglycerol benzoate from *Haloarcula japonica*.[4] It was initially suggested that the signals due to the two oxymethylene groups (3.50 and 3.65 ppm, Fig. 1C and 1D) of the phytanyl chains from methanogenic archaea were significantly reduced due to the corresponding glycerol signals and the deuterium being highly incorporated, *ca.* 90%. Since the spectra of biosynthesized lipids from methanogenic archaea (Fig. 1C and 1D) are similar to that of the halophile (Fig. 1B), it seems most likely that the saturation of a geranylgeranyl group in methanogen lipids take place similarly through the addition of hydrogens in a *syn*-manner. However, closer inspection of these spectra allowed us to immediately see the different proton incorporation between methanogen and halophile, which was unexpected, including the increased signal for the methyl groups (0.85 ppm) and the appearance of signals (1.05 ppm) in methanogen lipids. The latter signals at 1.05 ppm must be



Scheme 1 Structure of core lipids of the archaeal membrane.

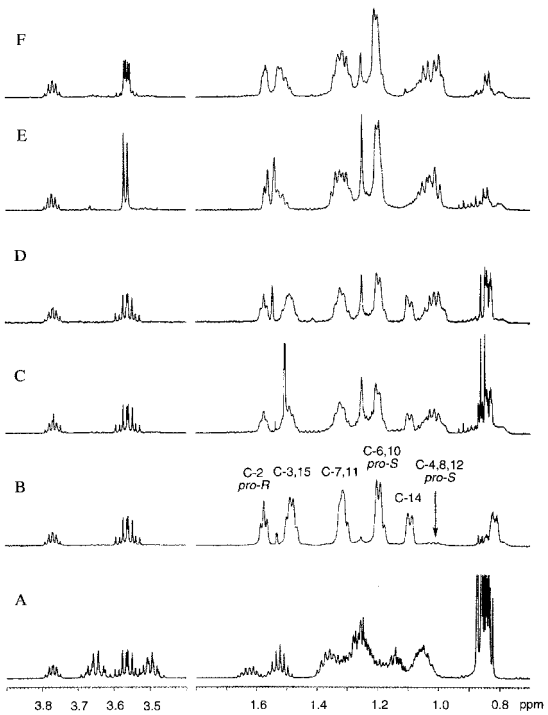
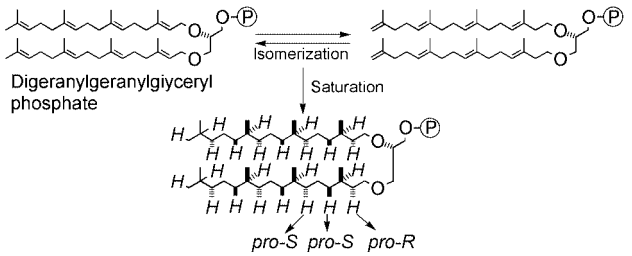


Fig. 1 Partial ^{1}H NMR spectra (500 MHz, $CDCl_3$) of (A) authentic 2,3-di-*O*-phytanyl-*sn*-glycerol benzoate, (B) multiply deuterated 2,3-di-*O*-phytanyl-*sn*-glycerol benzoate from *H. japonica* with mevalonolactone-d_9, (C) multiply deuterated 2,3-di-*O*-phytanyl-*sn*-glycerol benzoate from *M. thermoautotrophicum*, (D) multiply deuterated 2,3-di-*O*-phytanyl-*sn*-glycerol benzoate from *M. jannaschii*, (E) multiply deuterated 36-membered lipid benzoate from *M. jannaschii*, and (F) multiply deuterated 72-membered lipid benzoate from *M. jannaschii*.

† Electronic supplementary information (ESI) available: ^{1}H NMR and ^{1}H–^{1}H COSY spectra. See http://www.rsc.org/suppdata/cc/b0/b003948i/

ascribable to those of the diastereotopic protons of methylene groups at the C-4, -8 and -12 positions of phytanyl chains.[4] According to our previous assignment and the spectroscopic studies of others,[4,8] these signals were assigned to *pro-S* protons at the C-4, -8 and -12 positions. On the other hand, the signal intensities of the other positions were quite similar to those of the halophile. It now appears that the proton incorporation at these positions occurred regio- and stereospecifically in each phytanyl chain. This protonation pattern in these methanogen lipids cannot be rationalized by the well-known isomerase reaction between isopentenyl diphosphate and dimethylallyl diphosphate, because this isomerization reaction must cause stereochemical scrambling of protonation at the methylene group at the C-4, -8, and -12 positions of the phytanyl groups. Further, the increased methyl group signal at 0.85 ppm (Fig. 1C and 1D) can simply be assigned to the methyl groups at C-16 that originated from the C-2 position of mevalonate by ^{1}H–^{1}H COSY spectrum (data not shown), this was clearly verified by the reduced methyl signals of macrocyclic lipids (Fig. 1E and 1F), because the C-16 positions of macrocyclic lipids are known to originate from the C-2 position of mevalonate. Moreover, since digeranylgeranylglyceryl phosphate is known to be involved in the biosynthesis of archaeal lipids, this protonation pattern may well suggest involvement of migration of the double bonds, and this double-bond migration must occur *after* construction of the digeranylgeranylglyceryl group.

The present study now strongly suggests that the unusual double-bond migration occurred during the biosynthesis of the core lipid in the methanogenic archaea as shown in Scheme 2. Although it has not been rigorously verified, it is worth noting that the dissimilarity of the labeling patterns between methanogenic and halophilic archaea may reflect the presence and absence of macrocyclic lipids, and further, the isomerization of the double-bonds in digeranylgeranylglyceryl phosphate could be a trigger for new carbon–carbon bond(s) formation in macrocyclic lipids.



Scheme 2 Double-bond isomerization and saturation reaction in the lipid biosynthesis of methanogenic archaea.

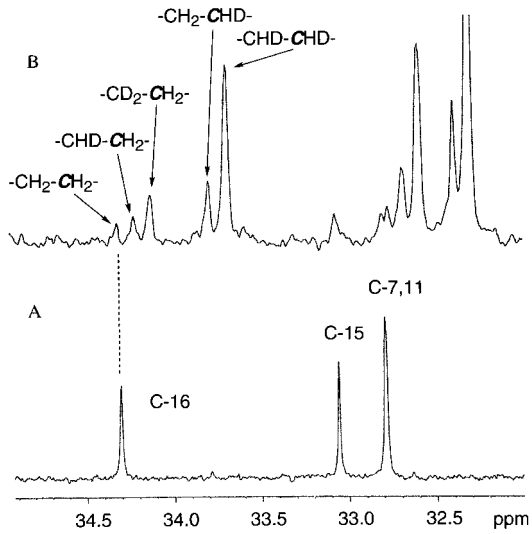
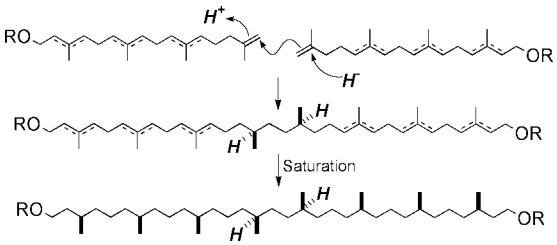


Fig. 2 Partial ^{13}C NMR spectra (125 MHz) of (A) non-labeled 72-membered lipid benzoate, and (B) multiply deuterated 72-membered lipid benzoate from *M. jannaschii* (^{1}H and ^{2}H decoupled).

Furthermore, the labeling patterns of biosynthetically deuterated 36- and 72-membered lipids were especially crucial in determining the nature of C-16, and the cyclized lipid benzoates were analyzed by ^{13}C{^{1}H,^{2}H} NMR spectroscopy. As shown in Fig. 2, the ^{13}C signals of the C-16 were observed as a cluster due to the various degrees of deuterium shift. Although the intensities of the cluster signals are low due to the isomerase reaction described above, a ^{13}C signal with two deuterium atoms retention was clearly observed at the C-16 position, which is a key feature in excluding higher oxidized states such as an aldehyde or a carboxylate as an intermediate for C–C bond formation.

These results further support the hypothesis that the isomerized intermediate having a terminal methylene functionality is a precursor. This precursor has to be ultimately saturated in the biosynthetic sequence. Saturation of isolated double bonds in various biological systems normally involves addition of a proton to generate a carbocation which is then quenched by hydride transfer from NAD(P)H. Therefore, a plausible and mechanistically simple solution to the problem of creating a new and critical C–C bond in the macrocyclic lipid biosynthesis is that the C–C bond formation occurs at the stage of the saturation step including intermolecular acid catalyzed condensation as shown in Scheme 3. While a mechanism involving a radical trigger can not be ruled out at the moment, the validity of this hypothesis is under investigation.



Scheme 3 Proposed mechanism of the new C–C bond forming reaction in the biosynthesis of macrocyclic lipids in methanogenic archaea.

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