Tightly Packed Membranes Composed of 36-Membered Macrocyclic Diether Phospholipid Found in Archaea Growing under Deep-sea Hydrothermal Vents

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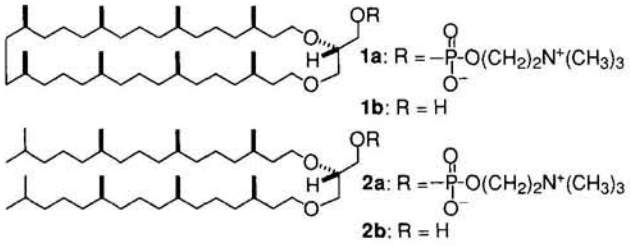
We were the first to investigate the properties of the archaeal 36-membered macrocyclic diether phospholipid **1a** in comparison with the corresponding acyclic counterpart **2a**. Results from thermal analyses and monolayer studies indicated that the 36-membered macrocyclic diether lipid can aggregate to more tightly packed structures than the acyclic congener due to less motional freedom at the alkyl chain region.

Archaea (archaebacteria) including thermophiles, halophiles, thermoacidophiles, alkalophiles and methanogens, which inhabit under extreme conditions such as high temperature, lack of oxygen, high acidic and/or salt-rich environments, have unique membrane lipids apart from those of eucarya and bacteria.[1-4] Archaeal lipids are typically composed of polyisoprenoid chains with ether linkage, and some methanogens and acidothermophiles have unusual macrocyclic lipids featuring 36- or 72-membered ring. Concerning to the role of these archaeal lipids, several model studies were reported in view of fluidity, permeability and thermostability of such lipids.[5-10] We have been interested in the biochemical significance of the archaeal macrocyclic lipids, and recently, total synthesis of the archaeal 36-membered macrocyclic lipid **1ab**, isolated from extreme thermophiles *Methanococcus jannaschii*,[2] has been reported.[11] In this paper, we wish to describe calorimetric and monolayer analyses of the membrane composed of the 36-membered diether phospholipid **1a**, in comparison with those of the corresponding acyclic diether lipid **2a** in order to elucidate the effects of macrocyclic structure in the archaeal membrane components.



The results of thermal analysis are summarized in Table 1. Aqueous dispersion of archaeal phospholipids **1a** and **2a** showed no obvious phase transition at a temperature ranging from -40 to 60°C. The branching methyl groups may cause lowering the phase transition temperature (T_c), and they must play a significant role in keeping the liquid-crystalline state with moderate fluidity at ambient temperature. In contrast, the corresponding core lipids **1b** and **2b** revealed endothermic phase transition at low temperature in the absence of water.[12] Furthermore, the cyclic core lipid **1b** showed higher T_c and lower phase transition enthalpy (ΔH_c) than the acyclic counterpart **2b**. These findings were similar to the results of Menger *et al.*, who used desmethylated model diether phospholipids[7b] and to our published results for the diether phospholipid analogues in aqueous media as well.[10] The present results appeared to show that macrocyclization of the alkyl chains caused three counts of effect: (i) elevation of T_c from **2b** to **1b** implies that the cyclic structure caused decrease of mobility, which resulted in demanding more energy to bring about the phase transition (order-disorder change), (ii) ΔH_c was lowered due to reducing the number of *gauche* C-C bonds, (iii) ΔS_c was also lowered by restricting the motional freedom of the alkyl chains. These results indicate that the covalent bond formation at the terminal of the isoprenoid chains causes the conformational change of the hydrophobic region from that of the non-bonded lipid, which may well affect molecular packed structures of the cyclic lipid.

Table 1. Phase transitions of the lipids[a]

Lipid	T_c [°C]	ΔH_c [kcal/mol]	ΔS_c [cal/mol·deg]
1a	not observed	-	-
2a	not observed	-	-
1b	-56.5	0.72	3.3
2b	-78.8	1.5	7.7

[a] Thermograms were run at a heating rate of 1 °C/min from -40 to 60 °C (for **1a** and **2a**), or at a heating rate of 2 °C/min from -120 to 0 °C (for **1b** and **2b**). Lipids **1a** and **2a** were dispersed in H_2O-ethylene glycol (4:1, v/v).

The π-A isotherms for monolayers of **1a** and **2a** on aqueous subphase are illustrated in Figure 2, and the deduced limiting area A_0 and the collapsing pressure π_c of these phospholipids are summarized in Table 2. The film area of cyclic lipid **1a** (0.70 nm^2/molecule) was significantly smaller than that of acyclic lipid **2a** (0.92 nm^2/molecule).[13] The cyclic lipid **1a** appears to aggregate into more closely packed structure than the acyclic lipid **2a** in the liquid-crystalline state.

Table 2. Characteristic data derived from π-A isotherms of the lipids **1a** and **2a**[a]

Lipid	Limiting area [nm^2/molecule]	Collapse pressure [mN/m]
1a	0.70	44
2a	0.92	52

[a] Each solution of lipid in 50 μl of $CHCl_3$ (1 mM) was gently dropped onto a trough (500x150 mm, filled with distilled water) at 20 °C. Monolayers were compressed at a rate of 0.32 mm/sec.

In conclusion, we first found that the covalent bond formation at the terminal of the isoprenoid chains in archaeal lipids forms more closely packed assemblies than the corresponding acyclic congener, thereby influencing the membrane fluidity. A tendency of the 36-membered lipid having more closely packed structures may well be related to the tolerance of *Methanococcus jannaschii* against high pressure and high temperature under deep-sea

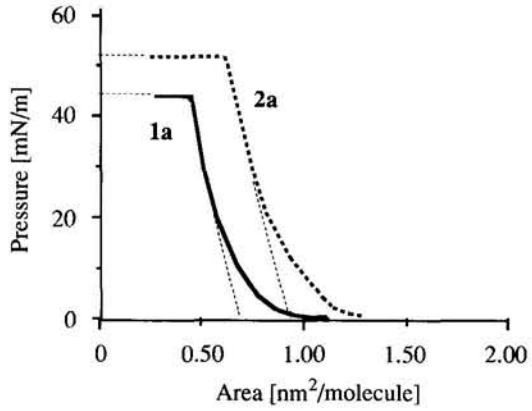


Figure 1. The π-A isotherms of the lipids **1a** and **2a**.

hydrothermal vents. Further studies on the properties of the 36-membered macrocyclic diether lipids in terms of thermostability and permeability are currently underway.

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12 Core lipids **1b** and **2b** are insoluble in water.

13 On our preliminary experiment using desmethylated model diether phospholipids,[10,11a] no apparent difference of the limiting area between the cyclic and the acyclic lipids was observed because of their solid state at ambient temperature.