Highly Thermostable Liposome from 72-Membered Macrocyclic Tetraether Lipid: Importance of 72-Membered Lipid for Archaea to Thrive under Hyperthermal Environments

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The thermostability of liposomes comprising from a 1:1 mixture of archaeal 72-membered macrocyclic tetraether diphospholipids was investigated for the first time in comparison with those of acyclic and 36-membered macrocyclic diether phospholipids. The liposomes of the former 72-membered macrocyclic lipid certainly form thermostable liposomes.

Archaea is classified as the third kingdom[1] and most of the archaeal microorganisms thrive under extreme conditions. A major characteristic of archaea is the chemical structures of membrane lipid. The archaeal membrane lipids are basically composed of rather regular isoprenoid chains tethered through ether linkages to glycerol at the *sn*-2 and -3 positions. A crucial feature of the lipids found in thermophiles and methanogens is the presence of macrocyclic structures, as large as 36- and 72-membered rings.[2] To clarify the role of these extremely macrocyclic lipids in thermophilic archaea, major synthetic and modeling efforts were reported[3] and various combinations of the mixed lipids of natural origin were examined.[4] Recently, we described the synthetic and characterization studies for these macrocyclic lipids.[5,6] As for the 36-membered macrocyclic lipid, **2** was found to form a less fluid, impermeable, and highly thermostable membrane in comparison with a corresponding acyclic congener **1**.[5d] More recently, we have reported for the first time the spontaneous vesicle formation of 1:1 mixture of the synthesized 72-membered macrocyclic tetraether diphospholipids **3a** and **3b** in aqueous media.[6b] Although these experiments were carried out by using a 1:1 mixture of **3a** and **3b**, the state of mixture was reasonable, since Arigoni et al. reported that the 72-membered lipids exist in several archaea as a mixture of regioisomers in terms of the glycerol arrangements such as **3a** and **3b**.[7]

In this paper, we describe the thermostability of vesicles composed of the mixture of 72-membered macrocyclic tetraether diphospholipids **3ab** in comparison with the diether phospholipid **1** and **2** to assess the significance of **3ab** in thermophilic archaea adapting to the hyperthermal environments.

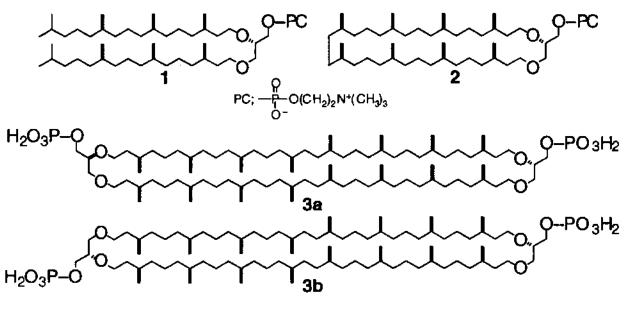

Figure 1. Structures of the synthetic archaeal lipids.

To estimate the thermostability, liposomes entrapping high concentration 6-carboxyfluorescein (CF) fluorophore[8] were prepared by a freeze-thaw method in a buffer (20 mM Tris-HCl, 200 mM NaCl, 200 mM CF at pH 7.5), followed by passage through a polycarbonate filter (ϕ = 200 nm) and a gel-permeation column.[9] The time-dependent CF-leakage profile from the liposome of **3** under various temperature were compared with those composed from the synthetic diether lipids **1**, **2**, and a polar lipid extract (PLE) of *Methanococcus jannaschii*,[10] and the results are shown in Figure 2 and are digitized in Table 1. The liposomes of the synthetic 72-membered macrocyclic lipids **3ab** showed a significant ability in retaining CF, particularly at elevated temperatures. The liposomes derived from **1** and **2** turned out to leak CF much easier at 50 °C and above, whereas the liposomes of **3ab** showed little leakage throughout the temperature tested. Clearly, **3ab** forms much more thermostable membrane by itself. The leakage extent of CF from the PLE liposomes ranged between those of **2** and **3ab** over the temperature tested. The results of the reconstituted PLE liposomes appeared to be mainly attributed to the existence of **3ab**, since the PLE is known to contain 43% **2**, 42% **3ab**, and 15% **1** with various polar head groups.[11] This observation was further supported by the fact that the content of **3ab** in PLE was increased by elevating the culture temperature.[11]

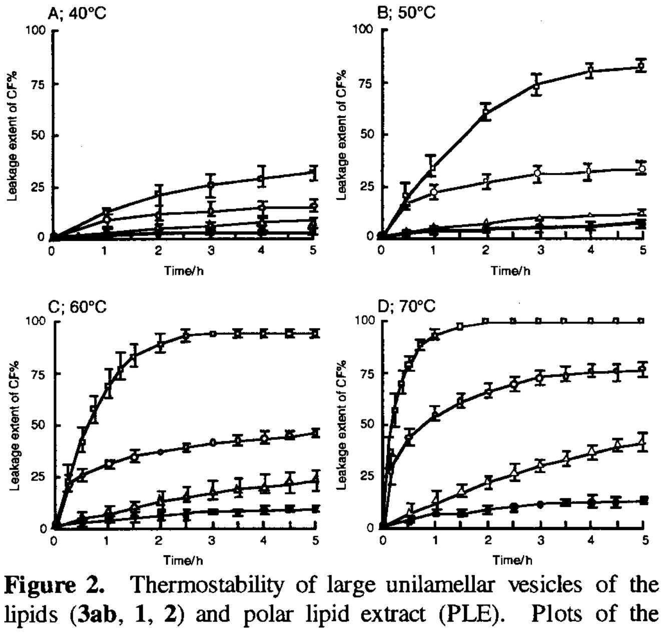

Figure 2. Thermostability of large unilamellar vesicles of the lipids (**3ab**, **1**, **2**) and polar lipid extract (PLE). Plots of the leakage extent of CF vs incubation time *t*. A; at 40 °C, B; at 50 °C, C; at 60 °C, D; at 70 °C; ●, **3ab**; ○, **2**; □, **1**; △, PLE.

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Table 1. Thermostability of liposomes

Lipid	Leakage extent(%)of CF after 5 h[a]			
	40 °C	50 °C	60 °C	70 °C
3ab	1.51	5.50	7.31	12.9
1[b]	31.2	83.5	94.0	100
2[b]	14.4	32.6	43.4	75.9
PLE[b]	7.99	11.8	23.0	40.6

[a]These values were average of at least three independent measurements. [b]See ref 5d.

The lipid **3ab** exists in the liquid–crystalline state at the temperature tested, because the phase-transition was not observed between 20–80 °C and the calorimetric measurements on core lipids of **3a** and **3b** indicated the presence of phase transitions at very low temperature in dry conditions.[6a] We reported that the macrocyclic structure in the 36-membered lipid **2** caused a more closely packed structure than the acyclic counterpart **1** by restricting the motional freedom of the alkyl chains in the liquid–crystalline state.[5d] This tendency of the macrocyclic lipids may be the case in the present study. Although the detailed polymorphism of 72-membered lipids **3ab** is not clear at the moment, these lipids can be expected, by virtue of their obvious amphiphilicity, to form organized systems in which the very long chain is extended across the membrane.[12] In this context, formation of multilamellar liposomes was previously reported with the naturally derived lipid mixture from *Sulfolobus acidocaldarius*.[13] The macrocyclic transmembrane structure should affect the rotational motions, and lateral diffusion of the lipids, which could as a result lead to stability of the membrane. Consequently, the 72-membered macrocyclic lipids **3ab** exhibit the highly thermal properties of its liposomes probably due to the presence of not only the macrocyclic structure but also the transmembrane structure, which may act to lower the membrane fluidity.

In conclusion, this present study clearly shows that the 72-membered macrocyclic tetraether lipids **3ab** are capable of forming the membranes that are stable under the temperature up to 70 °C. Therefore, the 72-membered lipid really plays an important role in the thermophilic archaea to thrive in extreme environments.

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