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Stability of Heterochiral Hybrid Membrane Made of Bacterial *sn*-G3P Lipids and Archaeal *sn*-G1P Lipids

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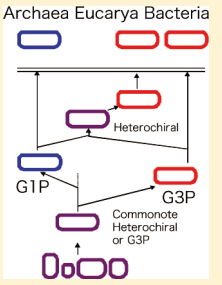
Supporting Information

ABSTRACT: The structure of membrane lipids in Archaea is different from those of Bacteria and Eucarya in many ways including the chirality of the glycerol backbone. Until now, heterochiral membranes were believed to be unstable; thus, no cellular organism could have existed before the separation of the groups of life. In this study, we tested the formation of heterochiral hybrid membrane made of Bacterial *sn*-glycerol-3-phosphate-type polar lipid and Archaeal *sn*-glycerol-1-phosphate-type polar lipid using the fluorescence probe. The stability of the hybrid liposomes made of phosphatidylethanolamines or phosphatidylcholines or polar lipids of thermophilic Bacteria and polar lipids of Archaea were investigated. The hybrid liposomes are all stable compared with homochiral liposome made of dimyristoylphosphatidylethanolamine and dipalmitoylphosphatidylcholine. However, the stability was drastically changed with increasing carbon chain length. Accordingly, "chirality" may not be but chain length is important. From these results, we suggest that the heterochiral hybrid membrane could be used as the membrane lipid for the last universal common ancestor (Commonote) before the emergence of Archaea and Bacteria.



Despite the argument on the universal ancestor based on the phylogenetic trees,[1−7] the consensus in the view of the universal common ancestor has not emerged. The universal common (cellular) ancestor has been called either LUCA,[8] LUCAS,[9] cenancestor,[10] progenote,[4] or Commonote.[2] All existing life is classified into three domains: Bacteria, Archaea, and Eucarya.[11,12] The membrane component before the emergence of Bacteria and Archaea is one of the foci of the argument[6,13−15] because membrane lipids that divide inside and outside of the cell are essential for life.

While various lipid structures are found in the three domains, they all have a glycerol backbone as a common structure, with the exception of the stereostructure. The stereostructure of the glycerol backbone in polar lipids of Bacteria and Eucarya is *sn*-glycerol-3-phosphate (*sn*-G3P, Figure 1a−c), while in polar lipids of Archaea, it is *sn*-glycerol-1-phosphate (*sn*-G1P, Figure 1d−f).[14,16] *sn*-G3P and *sn*-G1P are generated from dihydroxyacetone phosphate (DHAP) by different enzymes: *sn*-G3P dehydrogenase (G3PDH) and *sn*-G1P dehydrogenase (G1PDH), respectively. Since the encoding DNA and the deduced amino acid sequences of these enzymes have no similarity, Koga et al. proposed the hypothesis that the separation of Bacteria and Archaea might have been caused by cellularization by membranes with two enantiomeric lipids synthesized by G3PDH and G1PDH, which evolved from different enzymes.[14]

Martin and Russel proposed the hypothesis that Bacteria and Archaea emerged independently from the universal ancestor that was non-free-living cell in the iron monosulfide compartments.[17] On the hypothesis, Eucarya emerged from symbiosis of Archaea and Baceria and acquired the lipids from Bacteria.

Wächtershäuser also proposed a model[13] that incorporated the Koga model and the precell theory proposed by Kandler.[5] A precell is the state before the emergence of cells, metabolizing self-reproducing entities exhibiting most of the basic properties of a cell but unable to limit the frequent mutual exchange of genetic information. Wächtershäuser suggested the process from the precell to three stages: PC-1, PC-2, and PC-3. Although the precell had a membrane lipid with a heterochiral racemic glycerol backbone, it was slowly segregated to a stable homochiral membrane at an early point in the evolution of life. Bacteria emerged from PC-1 with the invention of G3PDH, and Archaea emerged from PC-2 with the invention of G1PDH. Subsequently, Eucarya emerged by a symbiosis between Bacteria and PC-3 with a predominance of *sn*-G3P type lipid enantiomers, avoiding the use of Archaeal lipids. Because PC-3 and Bacteria possessed a *sn*-G3P type membrane, the symbiosis progressed via the *sn*-G3P type homochiral membrane without using the unstable heterochiral membrane made of both *sn*-G3P and *sn*-G1P type lipids. However, these hypotheses are based on the postulation that the heterochiral membrane is unstable, and cells emerged after acquisition of the chiral selective enzyme. The heterochiral hybrid membrane has been assumed to be unstable and was expected to be spontaneously segregated.[13,14] This assumption was derived from the lipid incompatibilities that induce chiral discrimination.[18−20] Racemic mixtures of D- and L-myristoylalanine undergo chiral discrimination within a few minutes, followed by chiral segregation into D- and L-domains in ∼1 h.[18] On the other hand, the stability of a mixed membrane of Archaeal lipid and phosphatidylcholine has been investigated for the drug

Received: February 3, 2011
Revised: April 3, 2011
Published: April 08, 2011

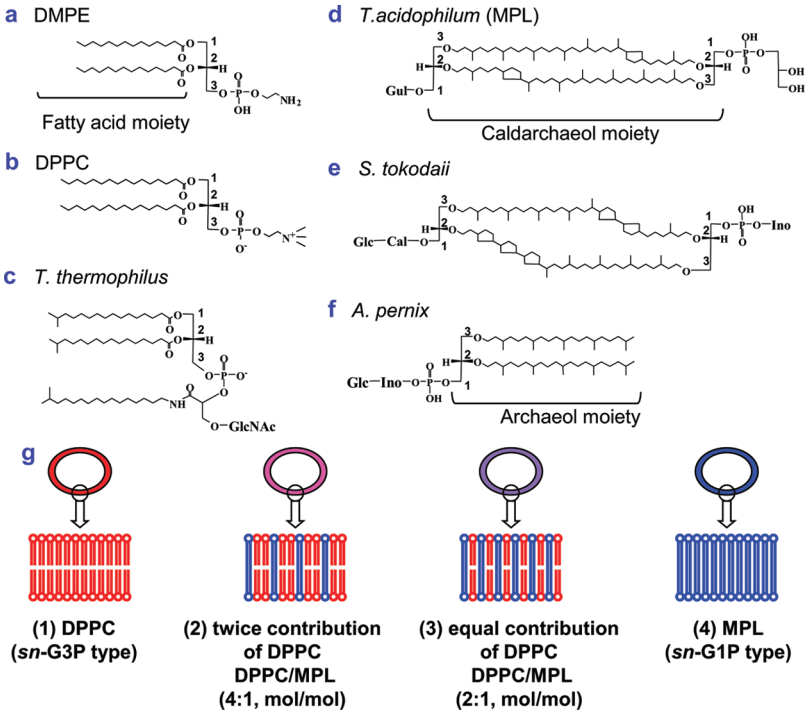


Figure 1. Structure of DPPC and one of the main polar lipids of thermophiles (a−f) and hybrid liposomes of DPPC and MPL of *T. acidophilum* (g). (a−c) DPPC and main polar lipid of a thermophilic Bacterium (*T. thermophilus*) have *sn*-G3P type glycerol backbone. (d−f) Main polar lipids of thermophilic Archaea (*T. acidophilum*,[29] *S. tokodaii and A. pernix*[35]) have *sn*-G1P type glycerol backbone. Structures of the polar lipids of *S. tokodaii* and *T. thermophilus* are summarized in Figures S2 and S3, respectively. Gul: L-gulose; Glc: D-glucose; Cal: calditol; GlcNAc: *N*-acetylglucosamine; Ino: mioinositol. (g) Arrangement of lipid molecules in membranes made of different ratios of DPPC and MPL. Molecular arrangement in the liposome membrane is schematically shown. Hydrophobic hydrocarbon chains (lines) are gathering at the center of the membrane, while polar moiety (circles) are exposed to the water phase. Molecules of DPPC and MPL are shown in red and blue, respectively.

delivery system. Fan et al.[21] reported that vesicles composed of mixtures of egg phosphatidylcholine and polar lipids from an extract of the Archaeon *Sulfolobus solfataricus* (at 2:1 molar ratio) were more stable than each liposome made of the respective polar lipid. In contrast, Sprott et al.[22] reported that the dilution of the polar lipids of *S. solfataricus* with egg phosphatidylcholine resulted in a decrease in the stability of the resultant liposomes. Another explanation for membrane stability was proposed based on molecular model calculations.[23] The driving force for phase separation in a monopolar−bipolar lipid mixture is the packing mismatch between the hydrophobic regions of the monopolar lipid hydrocarbon chains and the membrane-spanning bipolar lipids. A comprehensive understanding of the stability of mixed Bacterial and Archaeal lipid is still lacking. In this study, we prepared the hybrid membranes made of dimyristoylphosphatidylethanolamine (DMPE), a typical bacterial lipid, dipalmitoylphosphatidylcholine (DPPC), a typical Eucaryal lipid, or lipid from the thermophilic bacterium *Thermus thermophilus* with lipid extracts from various thermophilic Archaea. And the thermal stability of them was investigated.

EXPERIMENTAL PROCEDURES

Organism Cultivation and Extraction of Polar Lipids. *T. acidophilum* HO-62 was statically grown at 56 °C as described by Yasuda et al.[24] *Sulfolobus tokodaii* was aerobically grown at 80 °C as described by Inatomi et al.[25] *Aeropyrum pernix* K1 was aerobically grown at 92 °C as described by Sako et al.[26] *T. thermophilus* HB27 was aerobically grown at 70 °C as described by Tamakoshi et al.[27]

The total lipid extraction from each thermophile was described previously.[28] The total lipid was applied to a silica gel (Wakogel C200, 100−200 mesh, Wako, Osaka, Japan) column (10 × 100 mm) equilibrated with *n*-hexane. After the successive elution of neutral and low-polarity lipids with 40 mL each of *n*-hexane, chloroform, and acetone, the polar lipid was then eluted with 40 mL of chloroform/methanol (1:1, v/v). The main polar lipid of *T. acidophilum* (MPL) was purified from the polar lipid fraction as described previously.[29]

Liposome Preparation and Measurement of 5-CF Leakage. The polar lipid fraction of each thermophile, DPPC, and mixtures of appropriate molar ratios of the polar lipids of thermophilic Archaea and DPPC (or polar lipids of *T. thermophilus*) corresponding to 1 mg of lipid [dissolved in chloroform/methanol (2:1, vol/vol)] were put in a glass test tube. The solution was dried with nitrogen gas. A buffer solution containing 75 mM 5-CF, 5 mM potassium phosphate (pH 7.0), and 0.1 M potassium chloride was then added to the residue, and then the mixture was preheated at 70 °C for 30 min to hydrate the lipids. Liposome suspensions were prepared by sonication using a bath type ultrasonicator VS-100 (Asone, Osaka, Japan) from 0.5 to 5 min at 70 °C. The size of the liposomes was then adjusted to ∼0.1 μm diameter using a Mini-Extruder (Avanti, Alabaster, AL). The resulting vesicle suspension was passed through a Sephadex G50 column (Amersham Pharmacia Biotech UK Limited, Buckinghamshire, England) to remove the bulk of 5-CF outside the vesicle. A 5-CF-free buffer [5 mM potassium phosphate and 0.1 M potassium chloride (pH 7.0)] was used for elution. The orange color band containing the liposomes was

dx.doi.org/10.1021/bi200172d |*Biochemistry* 2011, 50, 4114–4120

then collected. The liposome suspension was then diluted with buffer (pH 7.0).

The fluorescence of 5-CF was measured using a spectrophotofluorometer RF-5300PC (Shimadzu, Kyoto, Japan). The 5-CF leakage was measured by the method described by Yamauchi et al.[30] and Choquet et al.[31] The fluorescence intensity of the suspension (I_{ini}) at 520 nm (emission bandwidth: 5 nm) with excitation at 470 nm (bandwidth: 3 nm) was measured at room temperature. To investigate the stability of the liposomes, 2.5 mL of the liposome suspension was incubated at the temperature indicated. After the measurement of fluorescence intensity (I_{aft}) of the incubated sample, 10 μL of 20% Triton X-100 aqueous solution was added, and the fluorescence (I_q) was measured. The leakage (%) was calculated by eq 1 shown in the Supporting Information.

Average Number of Cyclopentane Rings in Caldarchaeol Moiety of MPL. A caldarchaeol obtained by acid methanolysis of MPL was directly applied to the ELSD−HPLC system, and the average number of cyclopentane rings was calculated by the method described previously.[28]

RESULTS

Hybrid Liposomes Made of Bacterial/Eucaryal Lipid (*sn*-G3P Type Lipid) and Archaeal Lipid (*sn*-G1P Type Lipid). The generally occurring ester type monopolar lipids (*sn*-G3P type lipid, Figure 1a,b), which are found in Bacteria and Eucarya, form the bilayer membrane (Figure 1g(1)). In the membrane, polar moieties are exposed to the water phase, while hydrophobic hydrocarbon chains of fatty acid moieties gather at the membrane center. Some Archaeal species are known to possess totally different membrane lipids from those of the other life. The main polar lipid of the thermophilic Archaeon *T. acidophilum* HO-62, which is often called MPL, has a caldarchaeol moiety as the hydrocarbon core.[29] Cardarchaeol has two C40 hydrocarbon chains and two glycerol moieties (Figure 1d). The hydrocarbon chains are bonded at the *sn*-2 and *sn*-3 positions of the glycerol moieties to form the ether linkages, and the polar head groups are bonded at the *sn*-1 positions (*sn*-G1P type lipid, Figure 1d[16,32,33]). This bipolar lipid forms the monolayer membrane (Figure 1g (4)), instead of the generally occurring bilayer membrane (Figure 1g (1)).

A dipalmitoylphosphatidylcholine, DPPC, is *sn*-G3P type. Homochiral liposomes were made of DPPC and MPL, respectively. An equal contribution of DPPC and MPL was used for heterochiral hybrid liposome so that two kinds of lipids might influence in the maximum each other. One molecule of MPL corresponds to two molecules of DPPC, since MPL has a structure that the polar residues attached to both ends of the C40 hydrocarbon chain. To prepare a hybrid membrane with equal contributions of the *sn*-G3P type lipid and *sn*-G1P type lipid MPL, twice as many *sn*-G3P type lipid molecules are needed relative to the MPL molecules (Figure 1g (3)). We made hybrid liposomes composed of DPPC/MPL (2:1, mol/mol), and the thermal stability was investigated using 5-carboxyfluorescein (5-CF, as a fluorescent probe) entrapped in the liposome. When the 5-CF concentration is high, the fluorescent dye emits low fluorescence due to the self-quenching. The leakage of 5-CF can be estimated by monitoring the fluorescence intensity.[30,34] Because the common ancestor of all living organism may have been thermophilic or hyperthermophilic, the stability was tested at high temperature. Prominent leakage of 5-CF from the DPPC liposome was observed at 100 °C (Figure 2a). The liposome made of MPL showed much lower leakage of 5-CF under the same conditions. The leakage of 5-CF from the hybrid liposome made of DPPC and MPL (2:1, mol/mol) was even lower than the MPL liposome for 5 h (300 min).

The leakage from liposomes made of DPPC, the typical phospholipid of Eucarya, or DMPE, a typical lipid of Bacteria, and MPL, the typical phospholipid of Archaea, and their hybrids were tested at different temperatures (Figure 2b,c). If we assume the mixture of the liposomes, each made of DPPC or DMPC or MPL alone, the leakage of 5-CF from the liposomes made of mixtures of DPPC or DMPC/MPL (2:1, mol/mol), and those of DPPC or DMPC/MPL (4:1, mol/mol) could be calculated by a linear relationship. The expectations are shown as broken lines in Figure 2b,c. However, the leakage of 5-CF from the heterochiral hybrid liposomes was much lower than expected. The lipid compositions of the liposomes made of DPPC/MPL were investigated using HPTLC (Figure S1). The liposome still consists of DPPC and MPL. On the basis of these results, we can conclude that a stable heterochiral hybrid liposome made of *sn*-G3P type and *sn*-G1P type lipids was formed.

Stability of Hybrid Liposomes Made of DPPC or Polar Lipids of *T. thermophilus* and Polar Lipids of the Other Thermophilic Archaea. Hybrid liposomes made of DPPC and polar lipids of thermophilic Archaea were prepared so that the contribution of the Archaeal lipids was equal and 2/1 of DPPC (Figure 1g). Membrane lipids of *T. acidophilum* and *S. tokodaii* consist mainly of caldarchaeol type bipolar lipids (Figure 1d,e,[29] Figure S2). The molar ratio of DPPC and polar lipids of thermophiles equal to 2:1 and 4:1 were used for the preparation of the liposomes (Figure 2d,e). In contrast, *Aeropyrum prenix* has monopolar lipids with an archaeol moiety that is made of two C25 isoprenol chains attached to glycerol by an ether bond (Figure 1f[35]). Molar ratios (DPPC:*Aeropyrum prenix* lipids) of 1:1 and 2:1 were tested. The leakages of heterochiral hybrid liposomes were all lower than expected (Figure 2d−f).

T. thermophilus is a Bacterium whose optimum growth temperature is 65−72 °C[36] and has *sn*-G3P type polar lipids (Figure 1c, Figure S3). The homochiral liposome made of the polar lipids of *T. thermophilus* was stable, as was the liposome made of MPL (Figure 2g). The heterochiral hybrid liposomes made of the polar lipids of *T. thermophilus* and MPL (2:1 and 4:1 mol/mol) were stable under the test conditions. The liposomes were not only stable but also more stable than the homochiral liposomes under the conditions tested.

It has been reported that liposomes made of archaeol type monopolar lipids as well as normal ester type monopolar lipids are not stable at high temperature, but the liposomes made of caldarchaeol type bilayer lipids are stable.[22,37,38] However, liposomes made of monopolar lipids in this study, e.g., the polar lipids of *A. pernix* (archaeol (C25) type monopolar lipid) as well as those of *T. thermophilus* (Bacterial type monopolar lipid), were stable (Figure 2f,g). The caldarchaeol type lipid is not essential for the stability of liposomes. The monopolar lipids used in the previous report[22,37,38] were not obtained from thermophiles, while the monopolar lipids used in this study were obtained from the thermophiles (*A. pernix* and *T. thermophilus*). The membranes of thermophiles must be heat tolerant. The main polar lipids of *A. pernix* and *T. thermophilus* seem to have unique structural features (Figure 1f and Figure S3c): the molecular weight is comparatively large (more than 1000), and a sugar residue is present on the main polar lipids. These features are thought to be important for membrane stability at high temperature. Detailed

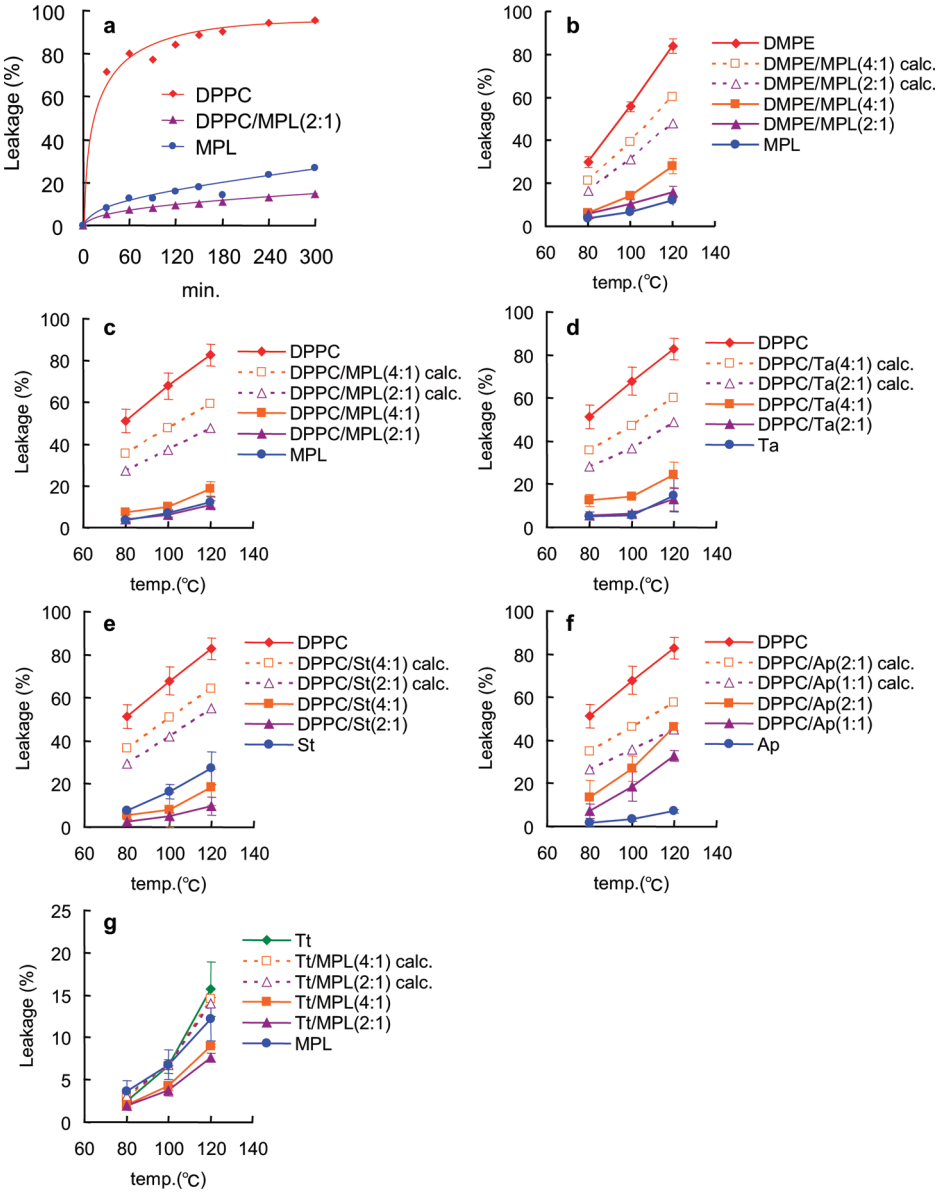


Figure 2. Stability of the heterochiral hybrid liposomes of DPPC or polar lipids of thermophilic Bacteria and polar lipids of thermophilic Archaea. (a) Time course of the 5-carboxyfluorescein leakage from the liposomes made of DPPC, DPPC/MPL (2:1, mol/mol), and MPL at 100 °C. (b–f) Temperature dependency on the 5-carboxyfluorescein leakage from the heterochiral hybrid liposomes: b, hybrid liposomes of DPPC and MPL; c, hybrid liposomes of DPPC and polar lipids of *T. acidophilum* HO-62; d, hybrid liposomes of DPPC and polar lipids of *S. tokodaii*; e, hybrid liposomes of DPPC and polar lipids of *A. pernix* K1; f, hybrid liposomes of the polar lipids of *T. thermophilus* HB27 and MPL: red ◆, liposomes made of DPPC; green ◆, liposomes made of polar lipid of *T. thermophilus* HB27; blue ●, liposomes made of polar lipid of thermophilic Archaea; purple ▲, hybrid liposomes made of equal contribution of the polar lipids of Bacteria and Archaea; orange ■, hybrid liposomes made of double contribution of the polar lipids of Bacteria against the polar lipids of thermophilic Archaea. A parenthesis shows the molar ratio of the polar lipids. The open symbols with broken lines show the expectation calculated from eq 2 shown in the Supporting Information. The experiments were repeated more than three times. Error bar shows standard deviation. The abbreviations of Tt, Ta, St, and Ap mean *T. thermophilus*, *T. acidophilum*, *S. tokodaii*, and *A. pernix*, respectively.

structural information is necessary to consider the results in the previous reports.

Effect of Hydrocarbon Chain Length in Phosphatidylcholine on the Stability of Hybrid Liposomes. To investigate the effect of hydrocarbon chain length, hybrid liposomes made of phosphatidylcholines consisting of various fatty acids with different carbon numbers and MPL were tested (Figure 3). The average number of cyclopentane rings in MPL was 2.9. The hybrid membrane made of MPL and DPPC and that made of MPL and distearoylphosphatidylcholine (DSPC) were most stable, while the hybrid membrane made of MPL and dilauroylphosphatidylcholine (DLPC), dimyristoylphosphatidylcholine (DMPC), or dibehenoylphosphatidylcholine (DBPC) were less stable. The stability of the hybrid membrane depended on the hydrocarbon chain length in phosphatidylcholines and was lower when the chain length was longer than C18 or shorter than C16.

■ DISCUSSION

It is thought that the lipids before the emergence of life were made nonenzymatically of *sn*-G1P, *sn*-G3P, and hydrocarbon and

dx.doi.org/10.1021/bi200172d |*Biochemistry* 2011, 50, 4114–4120

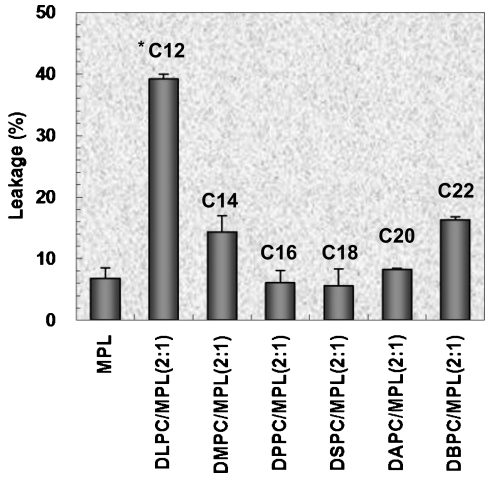


Figure 3. Effect of hydrocarbon length of phosphatidylcholine on the stability of hybrid liposomes at 100 °C for 30 min. Asterisk shows the carbon number of the hydrocarbon chains in phosphatidylcholine. DLPC = dilauroylphosphatidylcholine, DMPC = dimylistoylphosphatidylcholine, DPPC = dipalmitoylphosphatidylcholine, DSPC = distearoylphosphatidylcholine, DAPC = diarachidoylphosphatidylcholine, and DBPC = dibehenoylphosphatidylcholine. A parenthesis shows the molar ratio of MPL and phosphatidylcholines. The experiments were repeated more than three times. Error bar means standard deviation.

were racemic mixture.[13,16] Koga et al.[14] proposed a model that Archaea and Bacteria emerged independently upon acquisition of G1PDH and G3PDH, respectively, and possessed the homochiral membrane. Wächtershäuser has also proposed a model[13] that incorporated the model proposed by Koga et al. and precell theory proposed by Kandler.[5] These hypotheses were both based on the postulation that the heterochiral membrane is unstable, and cells emerged after acquisition of the chiral selective enzyme. The heterochiral hybrid membrane has been assumed to be unstable and expected to be spontaneously segregated.[13,16] This assumption was derived from the lipid incompatibilities that induce chiral discrimination.[18−20] Racemic mixtures of D- and L-myristoylalanine show the chiral discrimination in a few minutes, followed by chiral segregation into D- and L-domain in ∼1 h.[18] However, there is no structural similarity between amino acid in the lipid used in their experiments and glycerol backbone found in biological membrane lipid.

On the other hand, there are a few reports concerning the stability of the liposomes made of mixtures of Eucaryal lipid phosphatidylcholines and Archaeal lipid. Although Fan et al.[21] reported that the liposome made of mixtures of egg phosphatidylcholine and polar lipids extracted from *S. solfataricus* (at 2:1 molar ratio) was more stable than those made of Archaeal lipid alone, Sprott et al.[22] reported that the hybrid liposome is not stable but shows the intermediate stability between the liposome made of egg phosphatidylcholine and that made of Archaeal lipid.

In our study, the heterochiral hybrid membranes made of Eucaryal *sn*-G3P type lipid DPPC and archeal *sn*-G1P type lipid obtained form *S. tokodaii* were found to be stable at high temperatures (80, 100, and 120 °C), supporting the result of Fan et al.[21] We also found that the hybrid liposome made of DPPC and MPL, a purified *sn*-G1P type lipid, is stable at 100 °C for at least 5 h. The leakage rate at 100 °C was not increased for 5 h. If the chiral segregation had occurred in a few hours, the liposome predominantly made of DPPC would be rapidly broken, and the leakage would have accelerated. If the chiral segregation had been occurred by lateral diffusion, the membrane might be broken at the DPPC-rich area. The heterochiral hybrid liposome was stable without chiral segregation at least for 5 h. It was not only unstable but also more stable than DPPC and MPL homochiral membranes, respectively (Figure 2a). The heterochiral hybrid membranes made of bacterial *sn*-G3P type lipid DMPE and archeal *sn*-G1P type lipid MPL were also found to be stable at high temperatures (80, 100, and 120 °C). Other heterochiral hybrid membranes made of DPPC or the polar lipids of *T. thermophilus* and the polar lipids of thermopilic Archaea are also stable at high temperatures (80, 100, and 120 °C). The heterochiral hybrid membranes made of the polar lipids of *E. coli* and MPL were also stable at high temperature (Figure S4). These results show that the heterochiral hybrid liposomes containing the lipids of thermophilic Archaea are generally stable depending on the combination of lipids. It may be also important to note that the permeability of these heterochiral liposomes is within the range of permeability of liposomes made of natural lipids of these thermophilic organisms, suggesting that the heterochiral liposomes retain adequate permeability for sustaining life at high temperature.

While the chiral segregation was not detected, the stability of hybrid membrane was drastically changed with increasing carbon chain length of the fatty acid in phosphatidylcholine. The carbon chain length was expected to be responsible for the membrane stability, since it influences the packing of membrane lipids.[23] The number of cyclopentane rings in caldarchaeol of thermophilic archaeon is also responsible for the membrane thickness and packing of their membrane lipids.[39] The number of cyclopentan rings is known to change depending on the culture temperature.[28,40] When the number of cyclopentane rings in a caldarchaeol is 0−8, the membrane thickness from polar headgroup to polar headgroup is estimated to be 62.7−45.8 Å.[39] Since the average number of cyclopentane rings of MPL used in this study was 2.9, the membrane thickness is within the range. The thickness of the membranes made of DPPC and DSPC were estimated to be 51.3 and 57.2 Å, respectively.[39] The hybrid membrane made of DPPC or DSPC and MPL was stable probably because the membrane thickness of DPPC or DSPC was similar to those of MPL. Homochiral membranes made of DPPC and DSPC, however, were less stable than those made of the longer carbon chain lipids DAPC and DBPC (Figure S5). Though the homochiral membranes made of longer carbon chain phosphatidylcholines seems to be stable depending on the molecular weight, packing is more important for the stability of the hybrid membranes made of phosphatidylcholines and MPL. Chirality in the glycerol backbone is not important for the membrane stability. The discrepancy between the results reported by Fan et al.[21] and Sprott et al.[22] may have been caused by the difference in the lipid structures, e.g., hydrocarbon chain length of the phophatidylcholines, saturated or unsaturated hydrocarbon chain, and/or the different number of cyclopentan rings in caldarchaeol from *S. solfataricus*, although detailed structural information about the hydrocarbon moiety is not available. It remains unclear why the hybrid liposomes made of DPPC and a part of caldarchaeol type Archaeal lipids were more stable than the homochiral liposomes made of the corresponding Archaeal lipids. They might increase the rigidity, similar to the liposomes containing cholesterol.[41]

Since the heterochiral hybrid membrane made of *sn*-G3P type and *sn*-G1P type lipids was found to be stable, the hypothesis

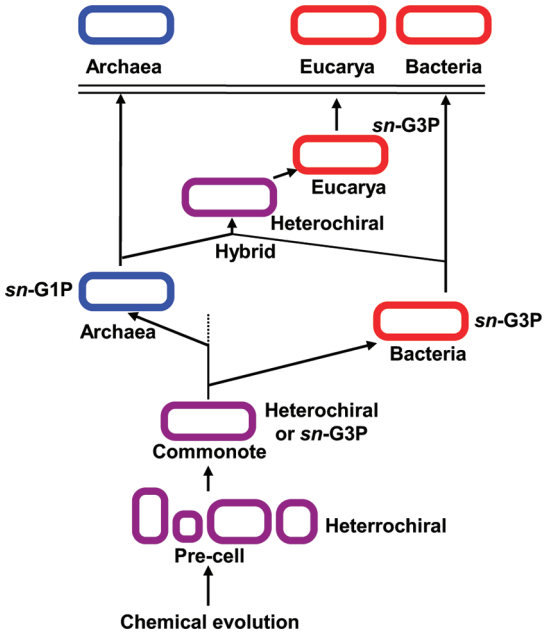


Figure 4. Model of the lipid composition changes at the early evolution of life. At the first stage at the early evolution of life, the various kinds of entities, Precell, would have evolved into the one living cell, Commonote; a common ancestor of life. These had a membrane without distinction of chirality shown in purple. After invention of G3PDH, Bacteria emerged from acquisition of G3PDH, and the membrane became *sn*-G3P type homochiral membrane shown in red. After invention of G1PDH, Archaea emerged from acquisition of G1PDH, and the membrane became *sn*-G1P type homochiral membrane shown in blue. Alternatively, the Commonote possessed both G3PDH and G1PDH, before the segregation of the lipids as well as the enzymes along with the emergence of Bacteria and Archaea. The third possibility is also possible, where the Commonote possessed either G3PDH or G1PDH, followed by the change of the membrane lipid to G1P or to G3P upon the replacement of the enzyme with G1PDH or G3PDH during the evolution toward Archaea or Bacteria, respectively. After Archaea and Bacteria diverged from the Commonote, Eucarya emerged from the Archaeal−Bacterial symbiosis or fusion.

"Bacteria and Archaea emerged independently" based on the assumption of the unstable heterochiral membrane loses its supporting evidence. The common ancestor of life before the emergence of Archaea and Bacteria (we call it the "Commonote"[2]) apparently was able to live without chiral segregation of lipids. Regarding the emergence of Eucarya based on an Archaeal−Bacterial symbiosis, no restriction is endowed by the compositional shift from *sn*-G1P type to *sn*-G3P type lipids, assuming the lipids have appropriate hydrocarbon chain lengths. Finally, we propose a model for the lipid compositional changes at the early evolution of life (Figure 4). Peretó et al.[6] have analyzed the phylogeny of enzymes relating to membrane lipids. They proposed the model of the evolution of lipid stereochemistry based on the phylogenetic analysis. In there model evolution of the two specific G1PDH and G3PDH activities by recruitment of dehydrogenases already present in the universal cenancestor would have been one of the triggering factors determining the speciation of Archaea and Bacteria (Figure 3 in ref 6). Our results regarding the stability of heterochiral membrane support their model and endow the model that universal ancestor with heterochiral membrane could have sufficient stability. Our results also supplement additional information regarding the membrane of common ancestor Commonote. (1) The heterochiral membranes are stable and not necessarily cause phase segregation. (2) Other cases, as described in the legend to Figure 4, where the cell of Commonote may be surrounded by homochiral membrane either of *sn*-G1P type or *sn*-G3P type lipid followed by the shift between these two lipids during the evolution toward Bacteria or Archaea are also possible. (3) Any change in chirality of the membrane is possible during the evolution from the Commonote to the contemporary organisms from the membrane stability point of view.

To conclude, we investigated the possibility of formation of heterochiral hybrid membranes composed of Bacterial and Eucaryal *sn*-G3P type polar lipids and Archaeal *sn*-G1P type polar lipids and investigated membrane stability. The heterochiral hybrid liposomes were stable compared to homochiral liposomes made of DPPC and DMPE. From these results, we suggest that the heterochiral hybrid membrane is as stable as the biomembrane of contemporary thermophilic microorganisms. These membranes could have been present at the early evolution of life as the membrane lipid components of the common ancestor Commonote before the emergence of Archaea and Bacteria.

■ ASSOCIATED CONTENT

Supporting Information. Supplementary figures and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

We thank S. Yokobori and M. Tamakoshi for the cultivation of *A. pernix* and *T. thermophilus* HB27. We also thank Y. Shida for MS measurements to reveal the polar lipid structures of *S. tokodaii* and *T. thermophilus* HB27.

■ ABBREVIATIONS

MPL, main polar lipid of *Thermoplasma acidophilum* HO-62; 5-CF, 5-carboxyfluorescein; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; *sn*-G1P, *sn*-glycerol-1-phosphate; *sn*-G3P, *sn*-glycerol-3-phosphate.

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